

Quantitation of Perchlorate Ion: Practices and Advances Applied to the Analysis of Common Matrices*

Edward Todd Urbansky

United States Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Water Supply and Water Resources Division, Cincinnati, Ohio 45268. E-mail: Urbansky.Edward@EPA.gov; Fax: 513-569-7658; Phone: 513-569-7655.

ABSTRACT: In 1997, low-level perchlorate contamination (<50 ng mL⁻¹ or parts per billion) was discovered in the western U.S. Since that time, it has been found in sites scattered around the nation. Although the Environmental Protection Agency has not established a regulation for perchlorate in drinking water, it has placed perchlorate on the contaminant candidate list (CCL) and the unregulated contaminants monitoring rule (UCMR). A provisional and unenforceable concentration of 18 ng mL⁻¹ will stand until at least late 2000 when EPA hopes to issue a revised toxicological assessment. However, the need for techniques and methods for determining perchlorate is not constrained to environmental chemistry. Perchlorate salts are used pharmaceutically in Europe to treat Graves' disease and amiodarone-induced thyrotoxicosis. Ammonium perchlorate is used as a solid oxidant in space shuttles and intercontinental ballistic missiles. Thus, methods and techniques are necessary for quality control and quality assurance. Moreover, analysis of explosives and post-explosion residues have made quantitation of perchlorate important in forensic chemistry. A variety of techniques is available: gravimetry, spectrophotometry, electrochemistry, ion chromatography, capillary electrophoresis, mass spectrometry—each has its strengths and weaknesses. Within each technique, assorted methods are available with corresponding limits of detection. As the breadth of matrices undergoing analysis expands from potable water to agricultural runoff, fertilizers, fruit juices, or physiological and botanical fluids, the risk for interference becomes greater. As toxicologists demand lower and lower limits of detection, it falls to analytical chemists to ensure selectivity and sensitivity go hand-in-hand. In the near future, we can expect refinements in sample pretreatment and clean-up as well as analytical methods geared toward analyzing more complex matrices. Ion chromatography, capillary electrophoresis, Raman spectrometry, and electrospray ionization mass spectrometry will all play roles in environmental analysis; however, IC should be expected to dominate drinking water analysis. This review describes the state of the science and how it might be applied, and looks forward to where it is going and how it might get there.

I. INTRODUCTION

Investigations of techniques and methods for quantitating perchlorate ion have been undertaken for several reasons. First, perchlorate salts, especially ammonium perchlorate, are used in missile, rocket, and space

shuttle propulsion systems. Second, perchlorate salts, usually KClO₄, have been used pharmaceutically. Third, electrochemical sensors are often evaluated in terms of Hofmeister behavior, for which perchlorate represents an extreme case. Fourth, and most recently, perchlorate has turned up in water

* Note: This paper is an original U.S. government work and is not subject to copyright.

supplies around the U.S. at $< 50 \text{ ng mL}^{-1}$ (ppb) concentrations. Fifth, perchlorate and similar oxyanions are seen in residues from explosions and there is a need to qualitatively and quantitatively analyze soil and dust for these analytes.

In the first two cases, there is a need for analysis to ensure quality control in production. Ammonium perchlorate is regarded as vital to national security. The importance of this chemical commodity can be inferred from what occurred after the Pacific Engineering and Production Company of Nevada (PEPCON) ammonium perchlorate plant exploded on May 4, 1988. PEPCON was one of two American production facilities at the time. Ammonium perchlorate is used in so many aerospace and military programs that it was questioned whether the government should have its own plants rather than relying on private suppliers. At the time of the explosion, U.S. government programs consumed 90% of the NH_4ClO_4 produced as more than 40 different weapon systems or aerospace programs relied on ammonium perchlorate.¹ Ammonium perchlorate continues to be important today in the propulsion sys-

tems of intercontinental ballistic missiles (ICBMs), such as the Peacekeeper missile (Figure 1), and the space shuttles.

With either chemotherapeutic use or drinking water contamination, there is a need to determine trace concentrations where perchlorate is a minor constituent relative to assorted organic and inorganic anions. At present, EPA's interest in analyzing surface, ground, and drinking water for perchlorate relates to possible health effects and the potential need for regulation. Many of the recent developments in perchlorate quantitation are a direct result of concerns over potable water. Beginning in 1997, perchlorate ion was discovered in natural waterways and aquifers around the western U.S.²⁻⁶ Affected regions include southern California (e.g., metropolitan Los Angeles), Nevada (especially greater Las Vegas), northwestern Arizona, and parts of Utah. This perchlorate problem is believed to be the legacy of years of legal dumping of wastewaters and most likely dates back several decades. The original salt was probably ammonium perchlorate, which continues to be used as a solid oxidant and energetics



FIGURE 1. The launch of an LGM-118 Peacekeeper missile, one of many U.S. defense systems that relies upon ammonium perchlorate. (Courtesy of U.S. Air Force.)

booster in missiles and rockets. Over time, ammonium perchlorate decomposes and must be replaced. The first step in this process is referred to as *hog out*—the removal of the old material. Some hog out sites as well as ordnance storage areas are known to suffer from perchlorate contamination.

Perchlorate is legendary in inorganic chemistry for its inertness to reduction despite the high oxidation state of the chlorine, +7; however, this behavior is due to kinetic reasons and not thermodynamic ones.^{2,3,7,8} Perchlorate's effects on human health and mechanism of action in the thyroid gland have been described elsewhere.^{2,4,9-11} The ion interferes with iodide uptake due to similarity in size and hydration and thus also interferes with thyroid hormone production. On account of the possible risks to human health through drinking water, EPA's Office of Water added perchlorate ion to the Contaminant Candidate List (CCL) in 1998¹²⁻¹³ and more recently to the Unregulated Contaminants Monitoring Rule (UCMR) in 1999.¹⁴

Although the analytical chemistry of perchlorate ion was briefly summarized in 1997,² the last 2 years have seen a dramatic increase in research, especially in that dedicated to trace quantitation in raw and finished drinking water supplies. While the area of greatest focus has been ion chromatography, developments have been made in other techniques as well. In particular, perchlorate quantitation in explosion residues has been an area of research in forensic chemistry. The primary focus of this review is on methods applicable to raw and finished potable water supplies; nevertheless, significant developments in other matrices have been made and are included as they may be directly applicable to water supplies or materials that have the potential to influence water supplies. It is worth pointing out that many samples can be converted to a dilute aqueous solution. Thus, techniques and methods developed for the analysis of aqueous solutions (e.g., drinking water) may be applied

to a variety of matrices that are themselves soluble or from which the ionic components may be leached.

II. GRAVIMETRY AND SPECTROPHOTOMETRY

For historical reasons, we first consider gravimetric and spectrophotometric determinations of perchlorate. The first attempt to review and evaluate the methods in the literature was undertaken by Lamb and Marden in 1912.¹⁵ The next major contribution was Nabar and Ramachandran's 1959 paper on the colorimetric determination of perchlorate with methylene blue.¹⁶ This set the foundation on which other authors would build future spectrophotometric methods. Several alternative classic methods preceded Nabar and Ramachandran, and these were summarized in their work.¹⁶ A notable exception was the gravimetric determination based on nitron, which is still in use today, but suffers from a variety of interferences as nitron precipitates many large anions, including perchlorate, iodide, nitrate, tungstate, bromide, and perrhenate.¹⁷⁻¹⁸ All of the analytical methods up to this point suffered from a wide variety of interferences as well as limitations in sensitivity. The lower limits of detection (LLODs) were generally inadequate.

After Wyngaarden et al.¹⁹ demonstrated perchlorate could be taken up by the thyroid gland in place of iodide in 1952, the interest in methods that could measure trace concentrations in physiological fluids was heightened. In 1968, Collinson and Boltz developed the first indirect atomic absorption method based on a perchloratocuprous complex.²⁰ This was the first reasonably selective method in the presence of common ions and had an LLOD of about 0.7 $\mu\text{g mL}^{-1}$. In 1972, Weiss and Stanbury modified Collinson and Boltz's method by employing ion exchange resins and applied the new

method to analyzing biological fluids (i.e., serum and urine).²¹ Subsequently, other developments were described in a previous review.² The last few years have seen additional work and that is covered here.

Burns et al. developed a method based on the extraction of an ion pair with protriptylinium cation.²² They include a useful review of spectrophotometric determinations. Their method suffers from interferences by permanganate, bromide, molybdate, thiocyanate, chlorate; however, evaporation with HCl alleviated these. The LLOD was 20 $\mu\text{g ClO}_4^-$. Thus, it would be necessary to evaporate off 4 L of water containing 5 ng mL^{-1} . In real water supplies, there are involatile organic anions that could substitute for perchlorate. Typical drinking water samples have $[\text{C}]_{\text{org}} = 300 \text{ ng mL}^{-1}$; thus, there would 300 μg of organic carbon for the 20 μg of perchlorate. If only 10% of it is ionic, the interference would still be severe.

Another recent method is analogous to the methylene blue method and is based on complexation with Astraphloxine FF and other dyes.²³ Typical of dye extraction-based methods, it was not very selective for perchlorate over other anions and λ_{max} is dye dependent. In the best case, $\epsilon = 1.22 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ (units not specified, but assumed); this suggests an LLOD of 0.24 $\mu\text{M} = 24 \text{ g } \mu\text{L}^{-1} = 24 \text{ ng mL}^{-1}$. In a slightly different twist, a chemoreceptor-dye interaction is disrupted by the perchlorate anion; this causes the absorbance change.²⁴ Unfortunately, nitrate and bromide are selected preferentially over perchlorate.

Ensafi and Rezaei developed a flow injection analysis method with automated extraction.²⁵ However, the method does not work in presence of chlorate, iodide, or nitrate. The authors report that heating to dryness with HCl eliminated these interferents, but they did not address the problem of involatile organic species. The LLOD was a promising 3 ng mL^{-1} . At 50 ng mL^{-1} perchlorate, the authors claim 500 ppm (10,000

times based on mass) chloride has no effect. Unfortunately, the sample preparation steps mean the method cannot be applied conveniently to drinking water because of chlorate and nitrate. Sample sizes of less than 1 mL are used; however, a fair amount of care is involved in the sample preparation. As a rule, interference and dye impurities would appear to pose high barriers to spectrophotometric or colorimetric methods at low (ppb) concentrations, but they might work in a QC/QA laboratory where the major anion in a material is perchlorate.

Of special interest is the possibility of spectrochemistry by design.²⁶ Hisamoto et al. synthesized selective dyes for use in optode (optical sensor) construction. These multiinformation dyes have not been applied to real matrices yet. Because selectivity is so important, molecular design and construction appear to be key areas of research if perchlorate is to be directly quantitated without separation whenever other common anions are present in high concentrations. Selectivity is still limited as illustrated in Figure 2, but this field is still developing. Although the authors did not test it, there is the potential for application to electrochemical sensors. A highly selective dye might be able to eliminate the selectivity problems associated with electrochemical detection without prior use of a separation technique (*vide infra*).

More promising than colorimetry or spectrophotometry is Raman scattering. The Raman phenomenon takes advantage of the unique changes in scattering frequencies associated with changes in the polarizability tensor (a function of the point group and the bond strengths).²⁷ Infrared spectrometry, which depends on changes in the dipole moment of the species during vibration, is hindered by the presence of water except in special applications (i.e., attenuated total reflectance or mirrored internal reflectance, which suffer from poor throughput). Unlike IR spectroscopy, Raman scattering is much less affected by water. In addition, molecules

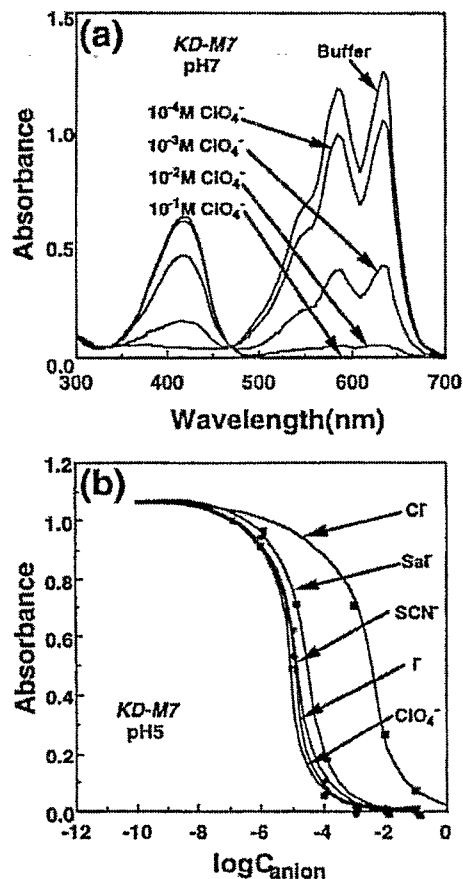


FIGURE 2. Typical characteristics of multi-information dyes used as optodes for perchlorate. Sal^- = salicylate. (Reprinted from *Anal. Chim. Acta* **1998**, 373, 271–289, with permission from Elsevier Science © 1998.)

and ions without net dipole moments can be Raman active even though they are IR inactive. Although perchlorate has no net dipole when tetrahedral, it can have a net dipole during bending, stretching, or wagging; therefore, it is in fact IR active. While Raman bands of solids tend to be sharper and better defined, this technique is readily applied to aqueous solutions. Generally, argon-ion or similar lasers are focused on a microscopic volume of solution. The light is scattered as it interacts with a perchlorate ion (or any chemical species) in solution. Scattered light with the same frequency as the incident light is called Rayleigh scattering (ν_0) and is not analytically significant. However, when the scattered light is shifted in frequency, this phenomenon is analytically significant. If

the ion gains energy from the light-particle interaction, the scattered photon has less energy than the incident photon ($\nu_0 - \delta$), and a Stokes band is seen. On the other hand, when the ion imparts energy to the photon ($\nu_0 + \delta$), an anti-Stokes band is seen. To minimize detection of Rayleigh and incident radiation, the detector is normally positioned perpendicular to the laser beam. Raman scattering can be used to definitively rule out the presence of perchlorate. Chlorine oxyanions have been studied by Raman spectroscopy in some detail and are well understood.²⁸ The Raman bands can be shifted by sodium or other cations, but this effect is systematic and is readily accounted for.²⁹ Although normally limited to minimum analyte concentrations of 1 to 10 mM, Raman spectroscopy

copy has been used successfully to measure as little as $1 \mu\text{g mL}^{-1}$ after preconcentration (Figure 3).³⁰ Most recently, it has been applied to aqueous leachates or solutions of fertilizers.^{31,32}

III. ELECTROCHEMISTRY

In this category, we consider recent developments in ion-selective electrodes (ISEs) as well as membrane field effect transistors (MEMFETs) and other sensors based on electricity. As noted above, perchlorate is used routinely in electrochemical studies as it occupies a key location in the Hofmeister series.^{33–35} Anions positions in the Hofmeister

series are generally considered to be a measure of Gibbs free energies of aquation and solubility of the cation-anion pair. The Hofmeister series for selectivity of an ion-selective electrode is as follows:³⁴ $\text{ClO}_4^- > \text{SCN}^- > \text{I}^- > \text{NO}_3^- > \text{Br}^- > \text{NO}_2^- > \text{Cl}^-$.

Many perchlorate ISEs are based on quaternary ammonium cations; these are heavily influenced by the solubilities of the perchlorate salts. More recent developments have concentrated on macrocyclic chemosensors or chemoreceptors.^{36–40} Some of the new electrodes demonstrate deviations from Hofmeister behavior (usually referred to as anti-Hofmeister even though the order is not necessarily reversed, but often jumbled).^{37–39} Macroscopic chemoreceptors used in some

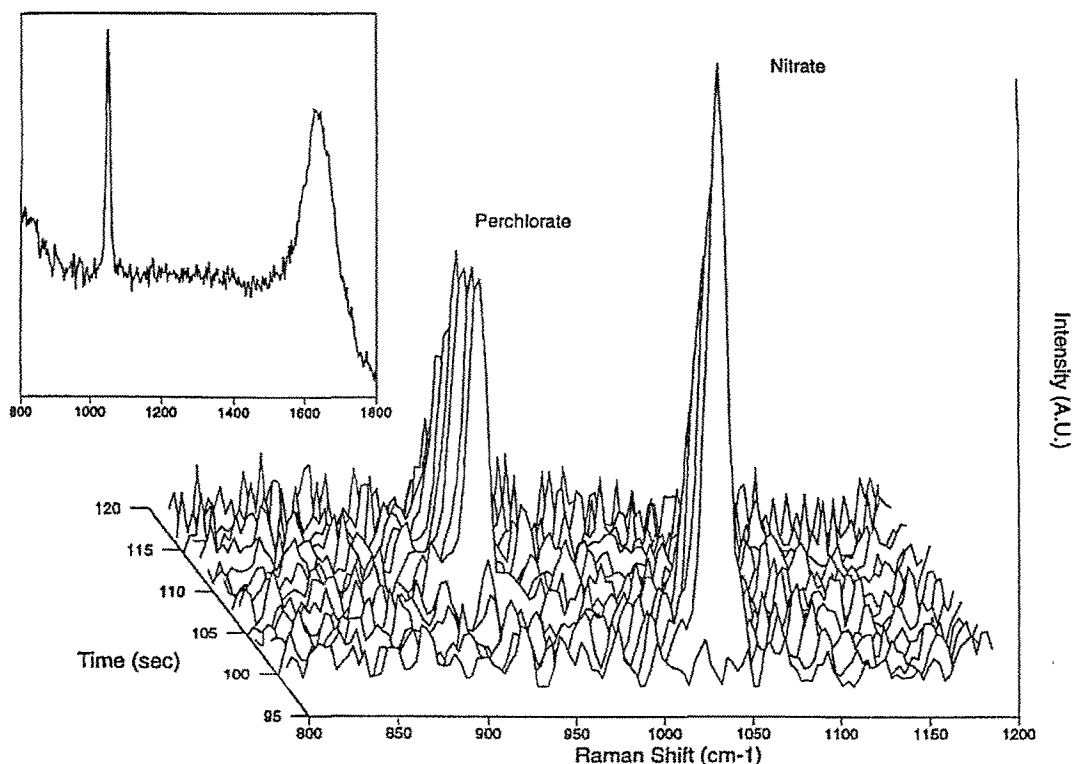


FIGURE 3. Raman spectra of nitrate (100 s, 1047 cm^{-1}) and perchlorate (107 s, 934 cm^{-1}) after electrophoretic separation. A Nd-Yag laser (532 nm) was used as the source; Rayleigh scattering was rejected with a holographic filter. Capillary: fused silica, coated with 3% T-linear polyacrylamide, 20 cm long \times 75 μm i.d. Conditions: 0.50 mM in each analyte; electrokinetic injection for 15 s at -2 kV . Spectra were flat-fielded and ratioed to the background electrolyte spectrum. A 5-point Savitsky-Golay quadratic smooth was employed. Inset is the raw spectrum at 100 s. (Reprinted from *Appl. Spectrosc.* 1995, 49, 1183–1188, with permission from the Society for Applied Spectroscopy © 1995.)

electrodes lead to deviations from Hofmeister behavior, and some electrodes response preferentially to iodide³⁶ or thiocyanate⁴⁰ over perchlorate.

A liquid membrane electrode based on Brilliant Green has an LLOD of $20\text{ }\mu\text{M}$ ($2\text{ }\mu\text{g mL}^{-1}$) for perchlorate.⁴¹ The most serious interferences come from periodate and permanganate. While neither of those is likely to be encountered in water, chloride (usually present at $\sim 3\text{ mM} = 100\text{ ppm}$) has selectivity coefficient about 10^{-6} that of perchlorate, but is present at a molar concentration about 10^6 times that of perchlorate.⁴¹ Another article by the same authors attempted to link potentiometric selectivity with partition coefficients.⁴² In it, they assumed 100% of precipitate formed by combining the cationic dye with the perchlorate was in fact the dye-perchlorate complex even though high salt concentrations were used to promote crystallization and the solid was not characterized.⁴² A selective fluorinated polyether (non-ionic) chemosensor for perchlorate reached a LLOD = $1\text{ }\mu\text{M} = 100\text{ ng mL}^{-1}$, even in high chloride (0.10 M).⁴³ Unfortunately, it still suffers from typical effects of nearby Hofmeister series anions.⁴³ Errachid et al.

wrote a solid paper on ionophore development and testing.⁴⁴ They produced MEMFETs and ISEs based on a phosphadithiamacrocycle. They saw well-behaved response (Figure 4) for perchlorate down to $1\text{ }\mu\text{M}$ and they obtained an LLOD of 100 nM .⁴⁴

Two papers were published on carbon paste electrodes.^{45,46} In the first case, an LLOD = $1\text{ }\mu\text{M} = 100\text{ ng mL}^{-1}$ was obtained, but interference problems would make it difficult to apply to drinking water matrices. It was nevertheless used successfully for cetylpyridium titrations to determine perchlorate in solution at higher concentrations.⁴⁵ In the second case, use of electrochemically generated thallium(0) to reduce perchlorate was applied to real tap water samples spiked with perchlorate. An LLOD of 50 ng mL^{-1} was obtained, but interferences from labile redox-active species (e.g., nitrate, nitrite, chlorate, bromate, arsenate, and arsenite) could confound the analysis.⁴⁶

One contribution was particularly novel and fascinating, but it is not ready for implementation. An electrochemiluminescent determination of perchlorate was reported in a recent communication by Xu and Dong.⁴⁷

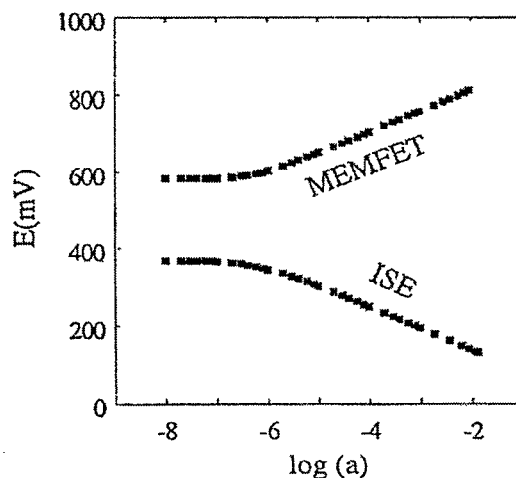


FIGURE 4. Perchlorate response for a membrane field-effect transistor (MEMFET) and ion-selective electrode (ISE) based on a phosphadithiamacrocycle chemosensor designed for use in PVC membranes. (Reprinted from *Sens. Actuators B* 1997, 43, 206–210, with permission from Elsevier Science © 1997.)

An LLOD of 50 nM (5 ng mL⁻¹) was obtained, but the method suffers from interference from iodide. Although iodide is not generally found in drinking water supplies, it would be present in the bloodstream and intracellular thyroid fluids. Incomplete information on interference from other anions precludes a thorough discussion of the applicability of this method for drinking water analysis. However, this appears to be the first attempt at assaying perchlorate by electrochemiluminescence, even though the Ru(bpy)₃²⁺ complex has been used for other analytes. Such combination techniques are probably necessary to obtain adequate sensitivity and selectivity.

At present, direct application of electrochemical methods is restricted to substances that are comprised primarily of perchlorate salts. Selectivity limitations make them likely to be applicable only to those solutions where it is already known that perchlorate is a predominant anion, as in a quality control laboratory or online, real-time process-monitoring system. In that capacity, they have rapid response, sufficient selectivity, and high sensitivity. However, interferences from other large, poorly aquated anions are severe enough to render these methods almost useless for typical drinking or raw waters. While selectivity for perchlorate relative to common ions such as chloride, bromide, sulfate, and nitrate is several orders of magnitude higher, the concentration of perchlorate is several orders of magnitude lower than the concentrations of the other ions. Curiously, the selectivity for perchlorate over other common anions is almost equally balanced by the concentrations at which these species normally occur. It is unclear whether standard additions could be used to vitiate matrix effects (primarily interferent anions). If a true blank could be measured by sequestering perchlorate with other reagents to account for the background signal of the interferents, then standard additions would permit broader application of electrochemi-

cal methods. This would require a highly selective sequestrant and would probably be difficult given the behavior of precipitants such as nitron or tetraphenylarsonium, which react with a wide variety of large anions. The candidates that immediately suggest themselves are Rb⁺ and Cs⁺; the perchlorate salts are sparingly soluble as perchlorate solubilities go (of course, there will be a counterion to balance the charge). Alternately, a carefully designed macro- or polycyclic molecule of the right size and shape might do the job as well. These areas of research remain unexplored. Despite these weaknesses of selectivity, highly sensitive electrochemical sensors may readily be applied if ions are separated before detection using ion chromatography or capillary electrophoresis, as we shall see in the sections that follow.

IV. CAPILLARY ELECTROPHORESIS

Capillary electrophoresis (CE) has been a rapidly advancing subdivision of separation science; more than 8000 papers using CE were published in 1999. This rapid proliferation of papers makes it almost impossible to keep up to date. At its most fundamental level, capillary electrophoresis is based on the differentiable migration of ions under the influence of an electric field. During its developmental phase, it was known by several synonyms, including capillary ion analysis, capillary ionophoresis, and capillary zone electrophoresis; however, the current usage favors capillary electrophoresis. The key term is capillary. Use of the capillary allows rapid analysis (<10 min) with a minimum of Joule heating (the effect of a current flowing through a salty solution). Readers unfamiliar with CE are referred to several books on the subject.⁴⁸⁻⁵¹

The discovery of trace perchlorate in ground and surface waters around California in 1997 is generally attributed to advance-

ments in ion chromatography that made low-level detection possible. However, Nann and Pretsch⁵² actually made the first report of determining perchlorate at 10 nM (1 ng mL⁻¹) in tap water by CE with an ion-selective microelectrode (ISME) in 1994 (Figure 5). In 1997, Ehmann et al. showed that CE could be used to determine 50 nM (5 ng mL⁻¹) perchlorate in the presence of 22 other equimolar anions (Figure 6).⁵³ In order to accomplish this, they used electrokinetic injection with isotachophoretic preconcentration. Unfortunately, both discoveries went completely unnoticed by the drinking water industry that has exclusively favored ion chromatography over CE (partly due to EPA Methods). Because many of the developments that take place in research laboratories are do-it-yourself and require time to construct, they are often overlooked or ignored by government and industry laboratories until they become commercially available.

Papers on CE can usually be divided into two groups: those that test new mobile phases

(also known as running buffer or background electrolyte solutions), often with a new chromophore or fluorophore, or those that test new detectors. CE itself is a separation technique and thus requires a detector. If the separation is sufficient, a fairly nonselective mode of detection may be used. Much of CE analysis is done using indirect detection, in which a loss of signal (e.g., fluorescence or absorbance) indicates the analyte is eluting in place of the background electrolyte. Direct detection by unselective electrochemical devices is also possible. Any measurable property of the analyte may be used for direct detection if the sensitivity of the detector is high enough.

Forensic CE has been applied to the analysis of residues from explosives by the Federal Bureau of Investigation (FBI)⁵⁴ and other law enforcement agencies.⁵⁵⁻⁵⁷ Hargadon and McCord report that recovered fragments of incendiary devices generally have sufficient residues remaining to meet the the 5 µg mL⁻¹ detection limit they report.⁵⁴ The FBI uses comparative analysis

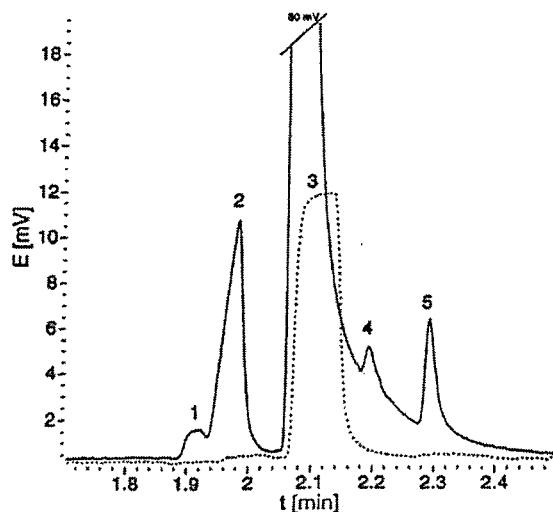


FIGURE 5. Electropherogram of 10 nM (1 ng mL⁻¹) perchlorate in tap water (solid line). Detection by ion specific micro electrode. Conditions: running electrolyte: 20 mM sodium hydrogen sulfate/sodium sulfate, pH 2.5; electrokinetic injection potential: 10 kV for 10 s; separation potential: 30 kV; uncoated capillary. Dotted line shows effect of adding 20 mM sulfate. Peaks: 1 = bromide, 2 = chloride, 3 = nitrate, 4 = perchlorate, 5 = unknown. (Reprinted from *J. Chromatogr. A* 1994, 676, 437–442, with permission from Elsevier Science © 1994.)

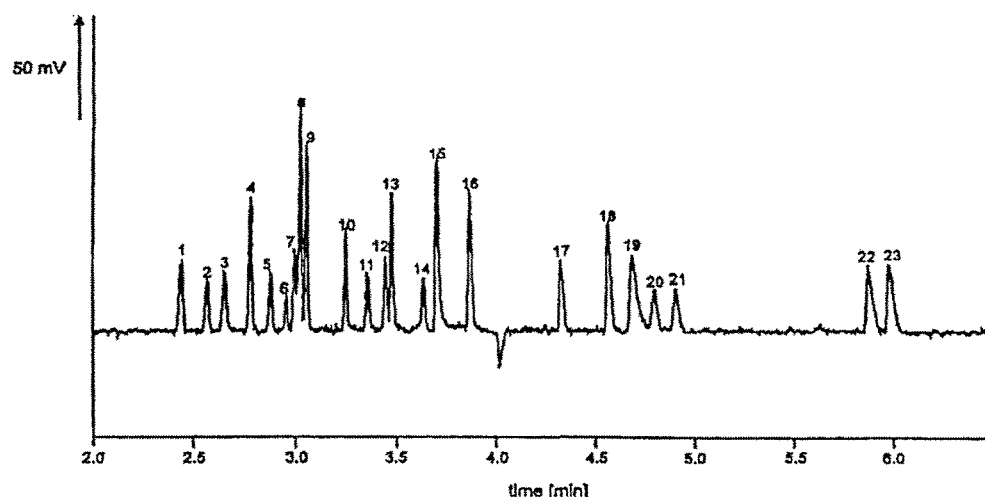


FIGURE 6. Electropherogram of 50 nM each on a Dionex CES 1 using electrokinetic injection and isotachophoretic preconcentration: Peaks: 1 = thiosulfate, 2 = bromide, 3 = chloride, 4 = sulfate, 5 = nitrite, 6 = tetrafluoroborate, 7 = nitrate, 8 = sulfosuccinate, 9 = oxalate, 10 = molybdate, 11 = perchlorate (5 ppb), 12 = thiocyanate, 13 = tungstate, 14 = chlorate, 15 = citrate, 16 = malonate, 17 = malate, 18 = tartrate, 19 = fluoride, 20 = bromate, 21 = formate, 22 = phosphate, 23 = arsenate. (Reprinted from *Chromatographia* 1997, 45, 301–311, with permission from Elsevier Science © 1997.)

with CE and IC to confirm identifications. Chromate is very popular for indirect detection in CE, and Figure 7 shows a typical result. Kishi et al. demonstrated that CE could be applied to other physical evidence, such as a cotton glove (Figure 8) used to handle fireworks.⁵⁵ Comparative analyses were also done by X-ray fluorescence spectroscopy and X-ray diffraction.

Using chromate as a chromophore can limit sensitivity with indirect detection. For that reason, a wide variety of chromophores, running electrolytes, and/or electroosmotic flow modifiers has been used (Figure 9), such as sulfosalicylate/imidazole (LLOD = 800 ng mL⁻¹),^{58,59} phthalate (LLOD = 600 ng mL⁻¹),^{60,61} indigotetrasulfonate (LLOD = 100 ng mL⁻¹),⁶² pyromellitic acid,⁶³ naphthalenesulfonates,⁶⁴ or cationic polymers.⁶⁵ Cyclodextrins can be used to change electrophoretic mobility and improve separation as shown in Figure 10.⁶⁶

Alternative detectors can improve sensitivity over that observed for indirect uv-absorbance. The use of conductivity for IC is common, and was applied to CE in 1993,

giving a perchlorate LLOD of about 10 ng mL⁻¹.⁶⁷ Fluorescence detection has been used⁶⁸ and is now commercially available. In 1999, Macka et al. reported on the use of a copper electrode for detection in CE.⁶⁹ This technology has not been applied to real samples yet and is not optimized, but initial work puts the LLOD at to 3 µg mL⁻¹.⁶⁹ Kappes et al. used coated-wire electrodes for detection.⁷⁰ Based on their electropherograms (Figure 11), perchlorate should have an LLOD of ~ 100 to 200 ng mL⁻¹. One advantage of the coated-wire electrodes over micropipet electrodes is their relative durability. Although substantial tailing is observed after 5 days, the coated-wire electrodes last 2 to 3 days.

Better techniques of sample volume reduction have allowed mass spectrometry (MS) to be used for detection, and CE-ionspray MS has been applied to solutions containing 1 mM perchlorate.⁷¹ Figure 12 demonstrates that the $m/z = 99$ u peak for perchlorate can be discriminated on the basis of m/z from the overlapping (coeluting) peaks of selenite, phosphite, and iodate.

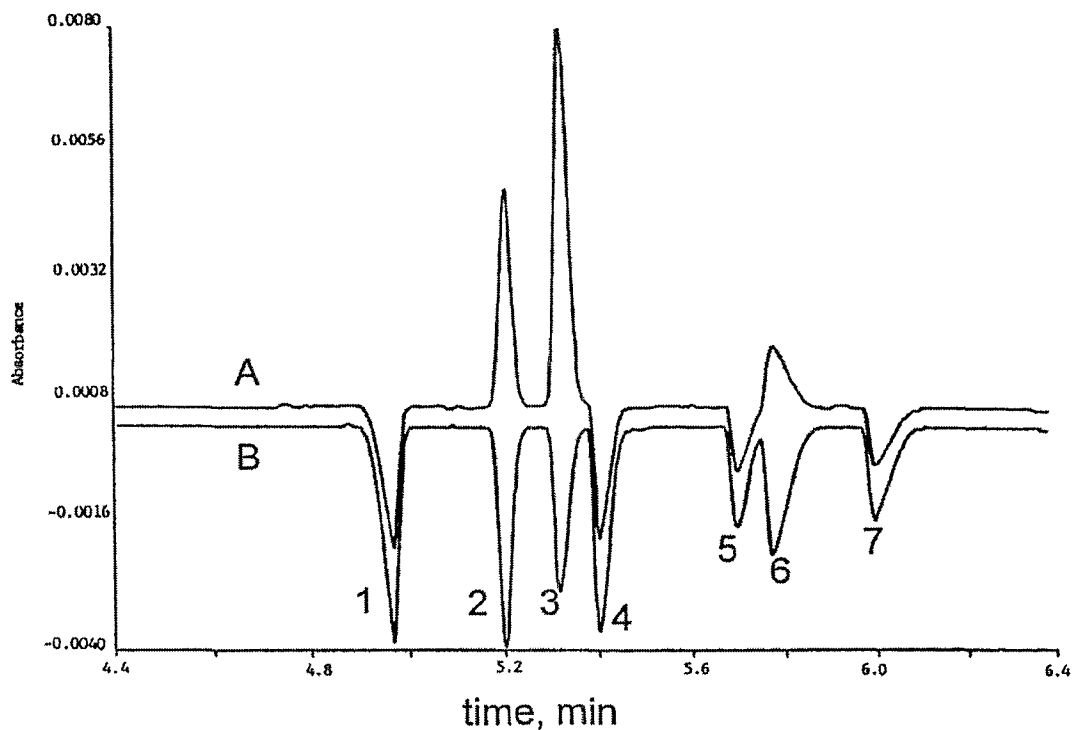


FIGURE 7. Electropherogram from the FBI Laboratory on a Dionex CES 1. Capillary: 65 cm \times 75 μ m i.d.; Conditions: 3.6 mM chromate, 1 mM diethylenetriamine, borate buffer, pH 7.8; hydrostatic (gravity) injection: \sim 50 nL; indirect uv-detection: A = 205 nm; B = 280 nm. Peaks: 1 = chloride, 2 = nitrite, 3 = nitrate, 4 = sulfate, 5 = **perchlorate**, 6 = thiocyanate, 7 = chlorate. Note that nitrite, nitrate, and thiocyanate all absorb at 205 nm, thereby producing positive peaks. This additional information can be used to confirm or rule out identities obtained by elution time using the signal at 280 nm. Concentrations not reported. (Reprinted from *J. Chromatogr.* **1992**, 602, 241–247, under U.S. government authority [not subject to copyright].)

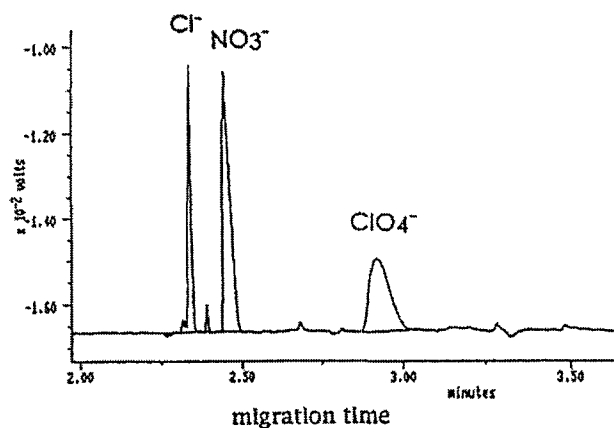


FIGURE 8. Electropherogram of an aqueous extract of a cotton glove used to handle fireworks. (Reprinted from *Electrophoresis* **1998**, 19, 3–5, with permission from John Wiley & Sons, Inc. © 1998.)

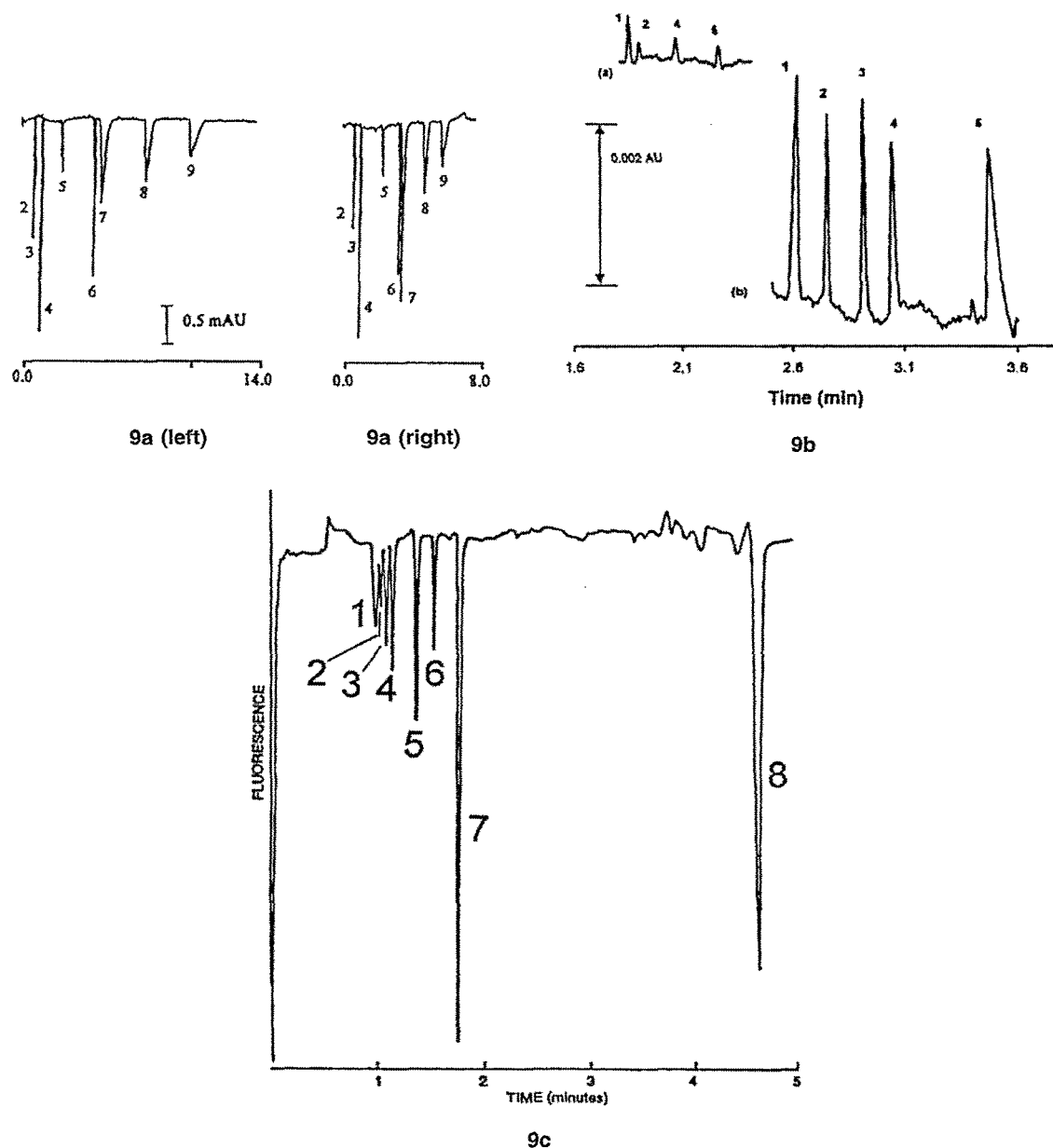


FIGURE 9. Electropherograms of mixtures of anions. (a) Capillary: 34.5 cm \times 50 μ m i.d. Conditions: 3.06 mM imidazole; applied potential: 15 kV; injection 15 s; current: 0.5 to 3.0 μ A; left: pH 6.52, right: pH 7.04; indirect uv detection at 214 nm. Peaks: 2 = sodium, 3 = lithium, 4 = water dip, 5 = phosphate, 6 = fluoride, 7 = formate, 8 = chlorate, 9 = perchlorate (8.7 g mL⁻¹). (Reprinted from *Electrophoresis*, 1998, 19, 2243–2251, with permission of John Wiley & Sons, Inc. © 1998.) (b) Inset conditions: 5 mM chromium (VI), 20 mM diethanolamine, 0.50 mM tetradecyl (myristyl) trimethylammonium bromide. Main figure conditions: 200 μ M potassium indigotetrasulfonate, 10 mM glutamic acid, 0.1% Carbowax, pH 3.22. applied potential: 30 kV; pressure injection: 0.6 s at 12.5 torr; indirect uv detection at 314 nm; 20 μ M each anion. Peaks: 1 = sulfate, 2 = nitrate, 3 = perchlorate (2 μ g mL⁻¹), 4 = chlorate, 5 = bromate. (Reprinted from *J. Chromatogr. A* 1998, 804, 327–336, with permission from Elsevier Science © 1998.) (c) Capillary: 51.2 cm \times 14 μ m i.d. Conditions: 0.25 mM salicylic acid/sodium salicylate; pH 4.0; electrokinetic injection for 0.7 s at 30 kV; current: 5.7 nA; applied potential: 30 kV; indirect fluorimetric detection using an argon ion laser. Peaks: 1 = chloride, 2 = nitrate, 3 = perchlorate (25 μ M = 2.5 μ g mL⁻¹), 4 = permanganate, 5 = dichromate, 6 = iodate, 7 = phosphate, 8 = salicylate. (Reprinted from *J. Chromatogr.* 1989, 480, 169–178, with permission from Elsevier Science © 1989.)

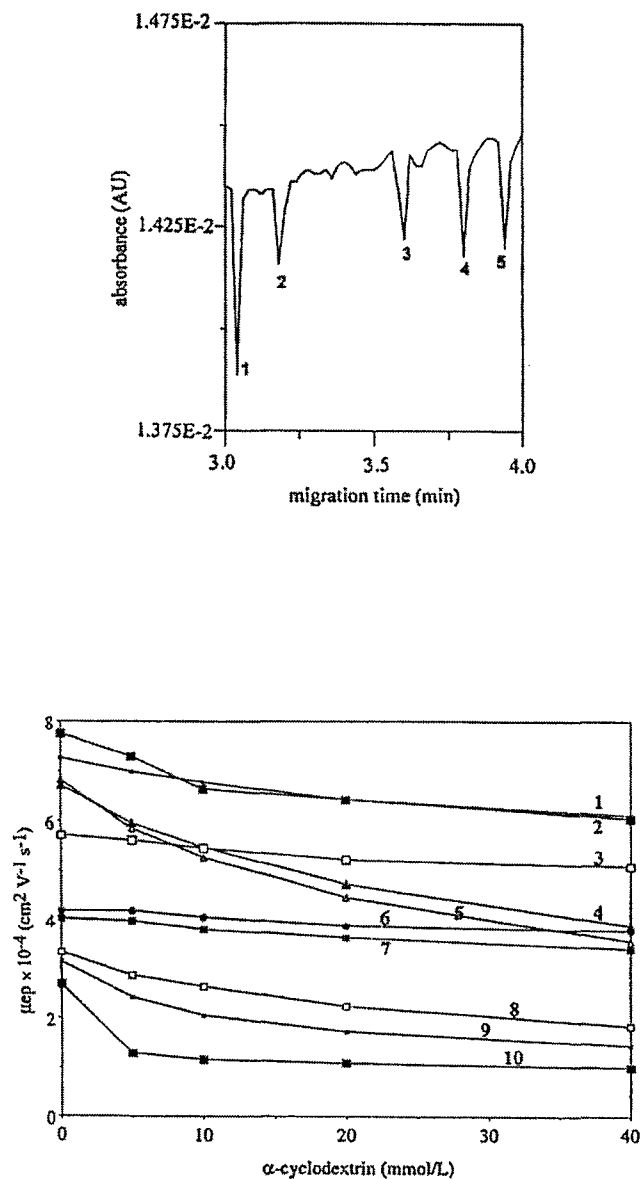


FIGURE 10. (A) Electropherogram of a mixture of anions (5 mM each) using α -cyclodextrin to affect ionic mobility. Conditions: 5 mM sodium chromate, 20 mM α -cyclodextrin, pH 8; hydrostatic injection for 15 s from a height of 10 cm ($\sim 100 \mu\text{M}$); applied electric field: 333 V cm^{-1} . Peaks: 1 = nitrate, 2 = iodide, 3 = bromate, 4 = thiocyanate, 5 = **perchlorate** ($500 \mu\text{g mL}^{-1}$). (B) Influence of α -cyclodextrin concentration on electrophoretic mobility. Conditions: 5 mM potassium hydrogen phthalate + 0.002% poly(1,1-dimethyl-3,5-dimethylenepiperidinium chloride) (molar mass = 200 kDa), as electroosmotic flow modifier. Line identifications 1–5 see (A); 6 = iodate, 7 = ethanesulfonate, 8 = butanesulfonate, 9 = pentanesulfonate, 10 = octanesulfonate. (Reprinted from *Can. J. Chem.* 1998, 76, 194–198, with permission from the National Research Council of Canada © 1998.)

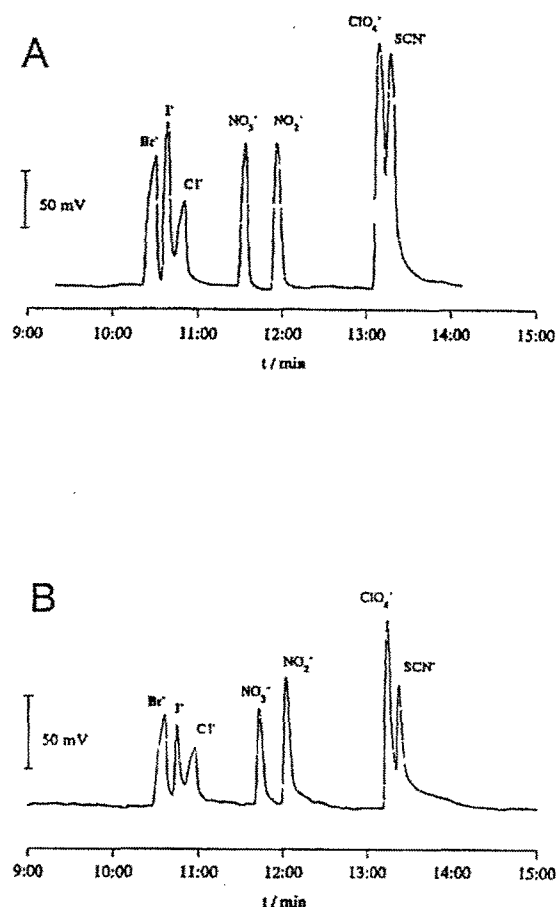


FIGURE 11. (A) Electropherogram of a mixture of anions. Capillary: 100 cm \times 25 μ m i.d., uncoated fused silica. Conditions: 10 mM potassium sulfate; electrokinetic injection for 7 s at -5.0 kV; applied potential: -30 kV. Concentrations: 10 μ M chloride, bromide, nitrite, nitrate; 2 μ M perchlorate, thiocyanate; 1 μ M iodide. (A) Using a micro pipet ISE based on a Mn(III) porphyrin (MnTPP). (B) Using a coated-wire ISE based on MnTPP. (Reprinted from *Anal. Chim. Acta* **1997**, 350, 141–147, with permission from Elsevier \copyright 1997.)

A variation of CE is capillary electrochromatography (CEC), which is included here because it operates under an applied electric field and can therefore take advantage of ionic (electrophoretic) mobilities. However, unlike CE, CEC relies on the types of secondary interactions between the analyte and the stationary phase that occur in any chromatographic process. In this respect, it is a hybrid of IC and CE because it uses the mechanisms of both. As shown in Figure 13, it has been applied to the determination of perchlorate at 50 mM (5 mg mL⁻¹).⁶⁰ Similarly, Hsu et al.⁷² modified the capillary wall by bonding a macrocyclic polyamine. The

polyamine structure is similar to an ampholyte and allows a continuous variation of electrophoretic mobility as a function of pH as shown in Figure 14. Hauser et al. modified a capillary with a polyacrylamide that incorporated a quaternary amine and added an ion-selective microelectrode, reaching 10 mM (1 mg mL⁻¹) as a detection limit for perchlorate.⁷³ One of the problems in CEC is reduced electroosmotic flow, as noted by Hilder et al., who used a Hypersil C₁₈ packing.⁷⁴ At first glance, there appears to be little benefit to using CEC over CE for quantitating perchlorate; however, CEC can better separate lipophilic species. Consequently,

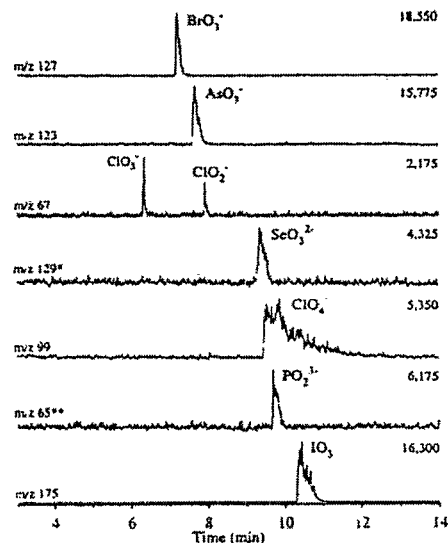


FIGURE 12. CE-ion spray-MS for inorganic anions (1 mM) using 2.5 mM pyromellitic acid in 20% MeOH, pH 7.8. *Singly protonated. **Doubly protonated. (Reprinted from *Anal. Chem.* 1996, 68, 2155–2163, with permission from the American Chemical Society © 1996.)

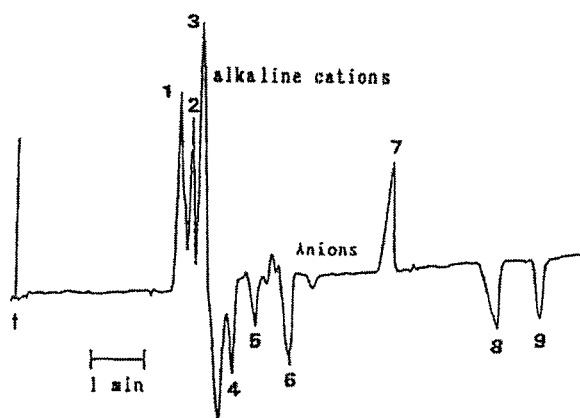


FIGURE 13. Electropherogram of a mixture of ions by capillary electrochromatography. Capillary: 30.4 cm long \times 50 μ m i.d.; packing: TSK IC-Anion-SW. Conditions: 90% v/v 5 mM phthalic acid and 5 mM hexamethylenediamine containing 0.15% HEPES, pH 6.8; 10% v/v MeOH; applied potential: 4 kV; indirect uv-detection at 236 nm. 1 = lithium, 2 = sodium, 3 = potassium, 4 = chloride, 5 = nitrite, 6 = nitrate, 7 = iodide, 8 = sulfate, 9 = perchlorate; concentrations not specified. (Reprinted from *J. Micro. Sep.* 1997, 9, 347–356, with permission from John Wiley & Sons, Inc. © 1997.)

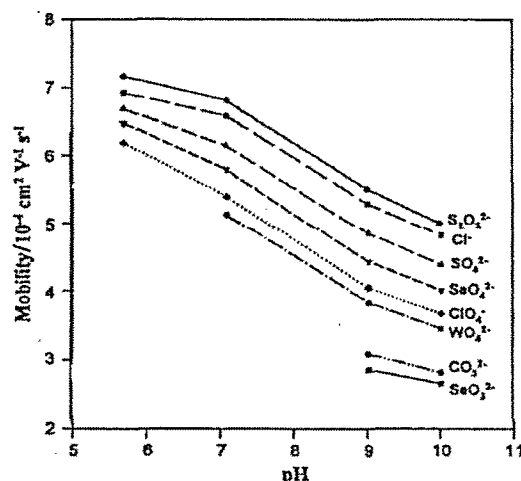


FIGURE 14. Electrophoretic mobility as a function of pH. Conditions: fused silica capillary covalently modified with [24]ane- $N_6 \times 50$ cm (100 μ m i.d.); 5 mM sodium chromate; applied potential: 15 kV; analyte concentration: 10 μ M (1 μ g mL $^{-1}$ for perchlorate); electrokinetic injection for 5 s at -15 kV; indirect uv detection at 250 nm. (Reprinted from the *Analyst* 1997, 122, 1393–1398, with permission from the Royal Society of Chemistry © 1997.)

it may be applicable to some samples of ground water, soil leachates, physiological fluids, and other matrices that potentially contain large amounts of soluble, polar, organic molecules (e.g., phenols, polyalcohols). Surface waters contain natural organic matter (humic and fulvic acids) at several ppm (as carbon), which commonly interfere in standard methods. CEC can simultaneously determine mixtures of hydrophilic and hydrophobic species, depending on the packing material used.

Meissner et al. used a related technique, isotachopheresis, to measure a series of anions.⁷⁵ In isotachopheresis, the terminal and leading background electrolytes are of low and high mobility, respectively (relative to each other and the analyte anions). The electroosmotic flow is often stopped by coating the capillary. As the anions migrate under the influence of the electric field, they are separated within the plug of injected solution.

The most widely available equipment and reagents for CE are presently restricted by LLODs of 100 ng mL $^{-1}$ (ppb) to 10 g mL $^{-1}$ (ppm). This makes them readily amenable to the analysis of explosives and other solid

materials where perchlorate anion is present at ≥ 1 mg kg $^{-1}$. Without using more sophisticated preconcentration steps or detectors, however, CE cannot reach the LLODs required for the routine analysis of potable water or raw waters, but could be used for some highly contaminated sites, where perchlorate concentrations in the parts-per-million range are common. Forensic analytical chemists have unquestionably demonstrated that CE is applicable to the determination of perchlorate in explosives and post-explosion residues. Therefore, we can expect to see CE play a role in that discipline. In those cases where perchlorate is much lower than other anions, peak overlap can be a serious problem in undiluted samples; however, dilution raises the net detection limit. Thus, there is an interplay between sensitivity of detectors and matrix effects that must be considered.

It is worth pointing out that the limits of CE with respect to analyzing water samples are related to practical sample size problems more than the sensitivity of available detectors. Indirect fluorimetric detection can reach down to the attomole (10^{-18} mol) region, which is fewer than 10^6 ions. Detecting 50 amol of perchlorate in a drinking water

sample containing 5 ng mL^{-1} would require an injection of 1 nL . In a capillary of $20 \text{ }\mu\text{m}$ i.d. that would be a 2.5-mm length of capillary filled with solution. Such a plug of solution would stop ionophoresis as it would limit the current. However, detecting 1 a mol would require only 20 pL , and thus only $50 \text{ }\mu\text{m}$ of the capillary to be used. Depending on the fluorophore, a few parts-per-billion is the practical lower limit of detection for CE. As pointed out by Nann and Pretsch,⁵² detecting an analyte present at 10 nM requires a detector capable of measuring about 20 zmol s^{-1} ($1 \text{ zeptomole} = 10^{-21} \text{ mol}$). Other than fluorescence, ISMEs and conductivity are adequately sensitive for analyzing potable water samples. Nevertheless, until such time as these devices are common on commercial instruments and make their way into methods approved by the EPA and other regulatory agencies, CE will continue to be displaced by IC for the determination of many ions found in drinking water and other liquid matrices.

V. ION CHROMATOGRAPHY

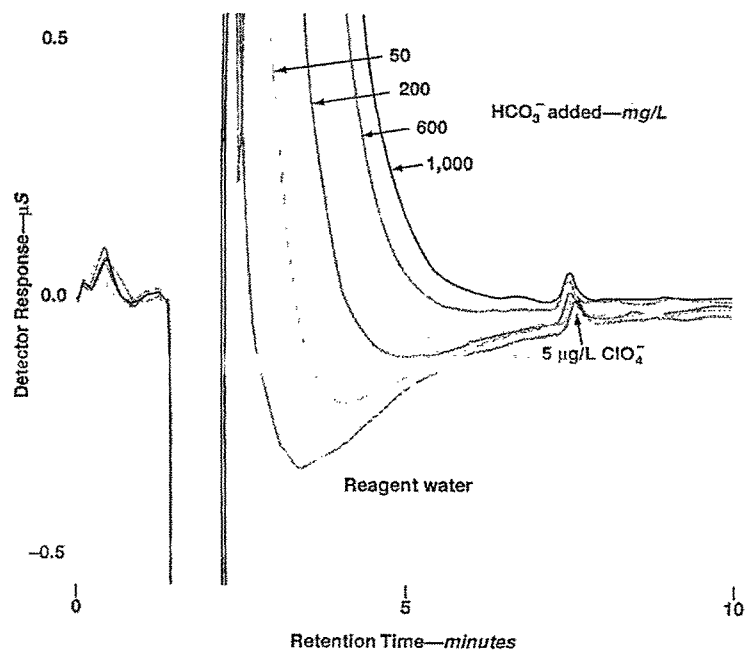
The fundamentals of ion chromatography (IC) are covered elsewhere.⁷⁶ In this review, we focus primarily on specific advances which make IC the technique of choice for drinking water at the present. Along with thiocyanate and iodide, perchlorate has a low charge density and is poorly aquated. Consequently, it is strongly retained on most columns and suffers from poor peak shape if it can be discerned eluting at all. Several actions can be taken to promote elution: (1) add an organic solvent (usually methanol) to the mobile phase,⁷⁷ (2) use a more hydrophobic anion in the eluent to displace the perchlorate,⁷⁸⁻⁸¹ (3) increase the concentration of the eluent anion, or (4) make the stationary phase more hydrophobic.⁸²⁻⁸⁵

Biesaga et al. illustrated the separation of perchlorate from other chlorine oxyanions

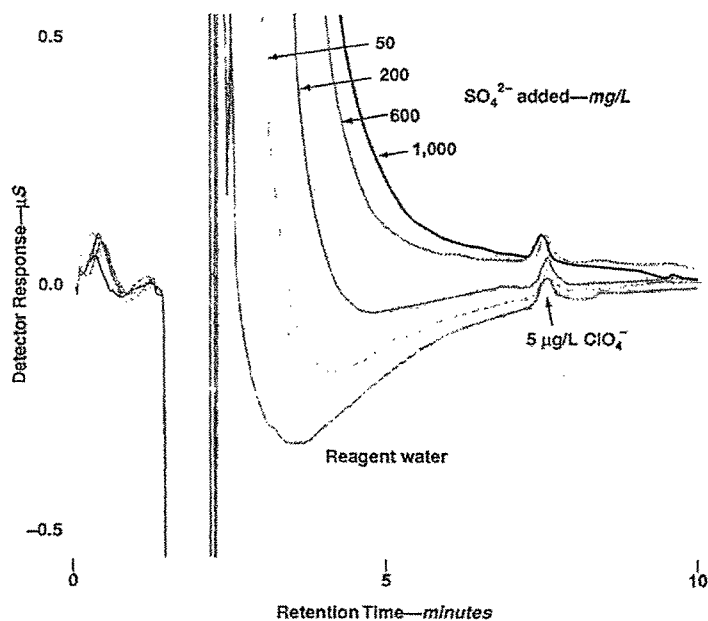
in tap water.⁶¹ The IC retention time was 30 to 40 min, but the peak was still well shaped. Unfortunately, the LLOD was $1.5 \text{ }\mu\text{g mL}^{-1}$. They obtained a CE LLOD of $0.6 \text{ }\mu\text{g mL}^{-1}$; at that time, CE outperformed IC. Phthalate was used as the eluent anion for IC. Relative to the concentration ranges that needed to be measured, this was inadequate.

One of the first breakthroughs was the development of a method based on *p*-cyanophenol/*p*-cyanophenoxide by Okamoto et al. at the California Department of Health Services in 1997.^{78,79} Miura et al. used 1,3,5-benzenetricarboxylate in the same way.⁸⁰ Also in 1997, Maurino and Minero were developing a method based on cyanuric acid/hydrogen cyanurate (cyanuric acid = 2,4,6-trihydroxy-1,3,5-triazine).⁸¹ Both Okamoto's and Maurino's method are based on hydrophilic columns (Dionex IonPac AS5 and AS4, respectively) using hydrophobic anions to displace the perchlorate. Okamoto et al. showed that satisfactory peak shape could be maintained with *p*-cyanophenoxide eluent even in the presence of high chloride, sulfate, and hydrogen carbonate. (Figure 15). The peak shape with *p*-cyanophenoxide is better than that obtained with cyanurate (Figure 16). In addition, Maurino and Minero reached an LLOD of $1 \text{ }\mu\text{g mL}^{-1}$, but Okamoto et al. were able to detect down to $\sim 1 \text{ ng mL}^{-1}$.

Because perchlorate is quite hydrophobic as anions go, Dionex has pursued use and development of columns intended to permit breakthrough of well-aquated ions (e.g., chloride, fluoride, bromide).⁸²⁻⁸⁵ Jackson et al. showed that the more hydrophobic IonPac AS11 column can be used with a large sample loop.⁸²⁻⁸⁵ The AS11 is limited in high sulfate⁸⁴⁻⁸⁶ as demonstrated in Figure 17; recoveries are unsatisfactorily low. Alltech has a methacrylate-based column for hydrophobic anions, but has not established methods that reach LLODs in the low ppb.⁸⁷ While the AS11 column can be used under appropriate conditions, the Dionex IonPac AS16 was developed specifically to take into



15a



15b

FIGURE 15. Ion chromatogram of perchlorate on a Dionex AG5 (guard) and AS5 (analytical) columns using *p*-cyanophenol/*p*-cyanophenoxide eluent. (A) Effect of hydrogen carbonate. (B) Effect of sulfate. (Reprinted from *J. Am. Water Works Assoc.* 1999, 91, 73–84, with permission from the American Water Works Association © 1999.)

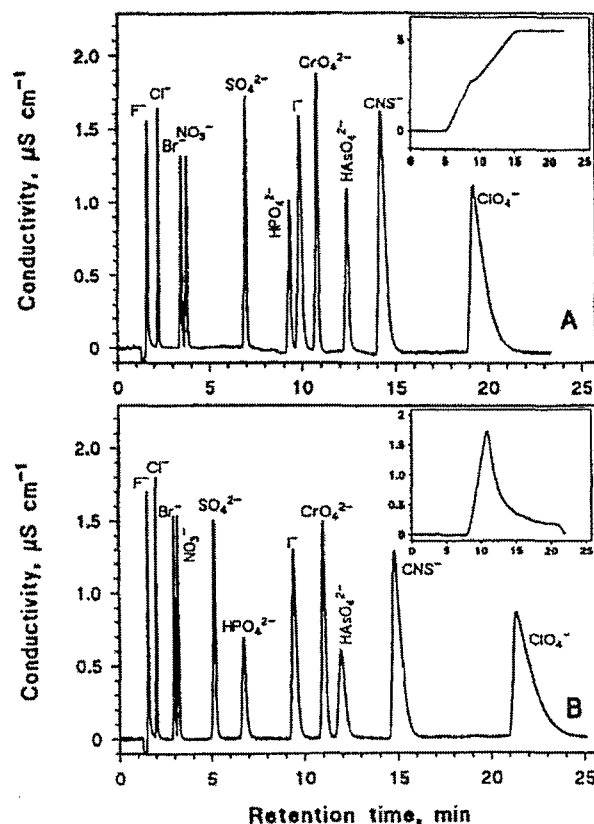


FIGURE 16. Ion chromatogram of perchlorate on a Dionex AG5 (guard) and AS5 (analytical) columns using *p*-cyanophenol/*p*-cyanophenoxide eluent. (A) Effect of hydrogen carbonate. (B) Effect of sulfate. (Reprinted from *J. Am. Water Works Assoc.* 1999, 91, 73–84, with permission from the American Water Works Association © 1999.)

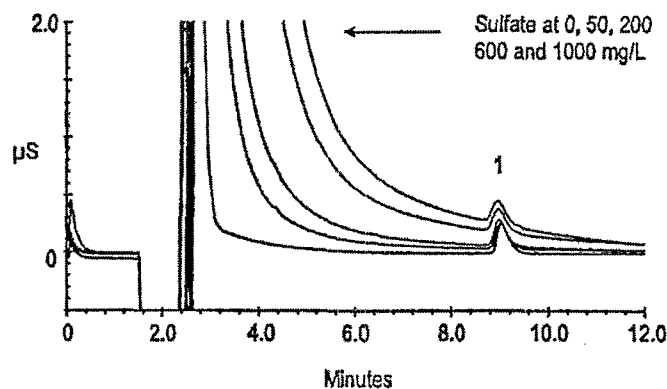


FIGURE 17. Ion chromatogram of 20 ng mL^{-1} perchlorate on the Dionex IonPac AG11 (guard) and AS11 (analytical) columns. Conditions: $0.10 \mu\text{M NaOH(aq)}$ eluent; flow rate: 1.0 mL min^{-1} ; suppressed conductivity; $1000 \mu\text{L}$ injection. Stacked chromatograms show the effect of adding sulfate (bottom to top) 0, 50, 200, 600, and $1000 \mu\text{g mL}^{-1}$. Peak 1 = perchlorate. (Reprinted from *J. Chromatogr. A* 1999, 850, 131–135, with permission from Elsevier Science © 1999.)

account the need for a column resistant to high concentrations of hydrophilic salts (e.g., NaCl, Na₂SO₄).^{84,85,88} Even in a high ionic strength synthetic ground water matrix, a well-shaped peak is obtained using the AS16 column (Figure 18). The U.S. Air Force Research Laboratory has actively pursued validation of the AS16 column under a wide variety of conditions.⁸⁹

Methods for the AS5 and AS11 are likely to be abandoned in favor of the AS16, which is the most resistant to common matrix effects. The Alltech methacrylate column is unlikely to play a major role unless further development is done to meet the needs of the drinking water industry. The Dionex IonPac AS16 column can be expected to dominate the analysis of drinking water, especially with the issuance of EPA Method 314,⁹⁰ which is expected to be promulgated for use in the UCMR. This resistance will be even more important as ion chromatography is applied to analyzing aqueous solutions of fertilizers, botanical fluids, and physiological fluids, all of which contain high concentrations of ions as well as assorted organic molecules that change the nature of the mobile phase or sorb to the column themselves.

The most common detector for ion chromatography is a conductivity cell, and it is the standard detector used on Dionex instru-

ments. However, conductivity is not selective; consequently, there will be a need for confirmation. Buchberger and Haider combined IC with particle beam mass spectrometry. Perchlorate was fragmented by electron impact to give 7 ions; no fragmentation was observed using chemical ionization.⁹¹ Corr and Anacleto also used MS for detection with IC the same as they did for CE.⁷¹

While the previous papers are very pragmatic and practical in nature—geared toward a very specific application, fundamental research in ion chromatography has not been neglected. A variety of properties and models has been used to explain the retention behavior observed for perchlorate. Daignault et al. attempted to correlate polarizability with the capacity factor (Figure 19).⁹² However, the formula used by Daignault et al. was taken out of context and misapplied. This does not entirely negate the relationship they saw, but still merits correction. The energy associated with London dispersion forces between two species A and B is related to the polarizability of the species by Eq. 1:⁹³

$$E \approx -\frac{3}{2} \left(\frac{I_A \cdot I_B}{I_A + I_B} \right) \left(\frac{\alpha_A \cdot \alpha_B}{R^6} \right) \quad (1)$$

where I represents ionization energy, α represents polarizability, and R represents the

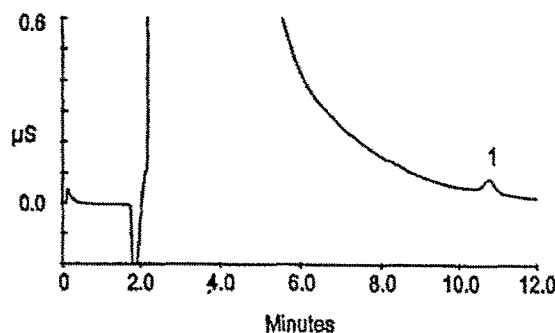


FIGURE 18. Ion chromatogram of 5 ng mL⁻¹ perchlorate in synthetic ground water on the Dionex IonPac AG16 (guard) and AS16 (analytical) columns. Synthetic ground water contains 200 µg mL⁻¹ chloride, 50 µg mL⁻¹ nitrate, 200 µg mL⁻¹ carbonate, 1000 µg mL⁻¹ sulfate. Peak 1 = perchlorate. (Reprinted from *J. Chromatogr. A* 1999, 850, 131–135, with permission from Elsevier Science © 1999.)

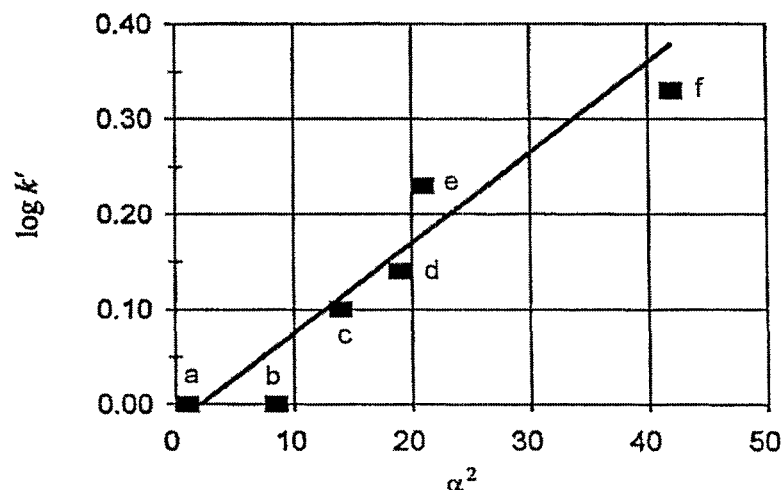


FIGURE 19. Log of capacity factor versus the square of the ionic polarizability. See text for additional detail. (Adapted from *J. High Resol. Chromatogr.* 1990, 13, 293–294, with permission from John Wiley & Sons © 1990.)

radial distance between the two species. However, when A and B are the same molecule or ion, Eq. 1 is reduced to Eq. 2.

$$E = -\frac{3I_A\alpha_A^2}{4R^6} \quad (2)$$

Daignault took Eq. 2 from Huheey et al.,⁹⁴ and is reminiscent of the attraction portion of the Lennard-Jones (6-12) potential, which applies to small nonpolar molecules. In an ion chromatography column, the retentive behavior is more likely to be related to the London forces between elements of the column (especially the quaternary ammonium moieties) and the anions rather than London attractive forces between the anions themselves. Accordingly, one might expect a relationship between $\alpha(\text{ClO}_4^-)$, $\alpha(\text{NR}_3^+)$, and k' . It is worth noting that the electrostatic force for two anions is repulsive, but this does not affect the London dispersion forces.

Martin studied the effects of organic solvents on capacity factors.⁹⁵ Adding acetonitrile, in particular, can reduce retention times to 1/3 their original value. Such behavior is not observed with methanol. Martin also considered the effect of an anion's hydration energy. The Dionex IonPac AS11

column was used for these experiments; thus, observations about the effects of MeCN, MeOH, or DMSO might be used to improve separations of refractory samples.

Several other studies examined fundamental behavior; however, a thorough analysis and interpretation is beyond the scope of this article. Okada modeled capacity factors in terms of the electric double layer theory to explain perchlorate's retention behavior.⁹⁶ Watanabe and Kubota had previously described this behavior in terms of streaming potential.⁹⁷ Pirogov et al. demonstrated that temperature dependence in retention behavior was similar for both methylmethacrylate and styrene-divinylbenzene polymer matrices.⁹⁸

A variety of studies have looked at alternate stationary phases. Takeuchi et al. showed that alumina could be used to separate perchlorate from chlorate, chlorite, and chloride.⁹⁹ Elkafir et al. used graphitic carbon to separate perchlorate from phosphate, sulfate, and nitrate with carboxylate-based eluents.¹⁰⁰ Muentner et al. used an 8-hydroxyquinoline-based stationary phase to separate perchlorate from chloride, bromide, and nitrate using a water/acetonitrile mobile phase.¹⁰¹ Based on electrochemical studies with cobalt(III)

phthalocyanines, Kocsis et al. incorporated these moieties into an octadecylsilane (C_{18}) column for use in anion chromatography; they separated perchlorate, nitrate, chloride, iodide, and thiocyanate using acetate as the eluent anion.¹⁰² Umemura et al. used octadecylsilane columns coated sulfobetaine surfactants to separate a mixture of anions, including chloride, bromide, perchlorate, chlorate, nitrate, iodide, thiocyanate, nitrite, and sulfate.¹⁰³ Pirogov et al. have tested a series of ionenes as modifiers for the mobile phase.¹⁰⁴ Ionenenes are polymers of the form $[NR_2^+-(CH_2)_a-NR_2^+-(CH_2)_b]_n$ and are named as ionene *a-b*. The quaternary ammonium moieties of the ionene mimic the functional groups of the stationary phase of a standard IC column. As Figure 20 indicates, excellent separation is possible with a standard octadecylsilane/silica stationary phase packing.¹⁰⁴ For now, these alternative stationary phases are developmental and unlikely to figure into routine monitoring anytime in the near future.

Ion chromatography is destined to be the main technique used for potable water in the U.S. for the foreseeable future. It couples low detection limits ($< 5 \text{ ng mL}^{-1}$) with ease of use, selectivity, and general availability. Low detection limits permit many samples to be diluted sufficiently to eliminate untoward matrix effects. IC tends to be rugged in a variety of matrices, and it has an established history in the drinking water industry in the U.S.

VI. MASS SPECTROMETRY

Because perchlorate is a small inorganic anion, certain ionization techniques suggest themselves: thermospray, ionspray, and electrospray (ESI-MS). Descriptions can be found elsewhere.^{105,106} While mass spectrometry can be used as a detector subsequent to separation by IC or CE, it is possible to perform mass spectrometric analysis of a

sample without separation. Barnett and Horlick used ESI-MS to obtain an LLOD of 5 ng mL^{-1} for perchlorate.¹⁰⁷ They examined solutions of quaternary ammonium compounds (mouthwashes) for perchlorate. At the U.S. Air Force Research Laboratories, Clewell et al. lyophilized water samples and redissolved the residue in acetonitrile/acetic acid.^{108,109} Figure 21 shows a mass spectrum of the perchlorate anion ($m/z = 99$ u). One advantage to this approach is that carbonate is driven off. They obtained a detection limit of 340 pg mL^{-1} . Typical negative ion ESI-MS response is shown in Figure 22 for several concentrations of perchlorate (single ion monitoring).

At the EPA, Urbansky et al. showed that perchlorate could be complexed with nonnucleophilic bulky organic bases (diazabicyclo compounds) to give molecular ions of the form $HB(CIO_4)_2^-$; the best sensitivity was obtained with chlorhexidine, which is a minimally nucleophilic base.¹¹⁰ This phenomenon was further exploited by Magnuson et al., who demonstrated that perchlorate could be extracted into methylene chloride with quaternary ammonium cations (e.g., $C_{10}H_{21}NMe_3^+$); see Figure 23.¹¹¹ The concentration of perchlorate in real water samples was determined by standard additions using ESI-MS with an LLOD of about 30 to 300 pg mL^{-1} . The matrix can affect the sensitivity, but the variation was less than a factor of 10 among tested water samples (Figure 24). This work was followed by the development of a microextraction procedure¹¹² and an alternate procedure using methyl isobutyl ketone (MIBK).¹¹³ The LLOD in MIBK is about 5 ng mL^{-1} or 10 to 100 times that in CH_2Cl_2 . The MIBK method was applied to the analysis of bottled waters, many of which contain high concentrations of dissolved minerals. Although sparkling mineral waters do not normally pose serious problems for IC, they can be refractory to ESI-MS methods, presumably due to competition for the cationic surfactant and/or

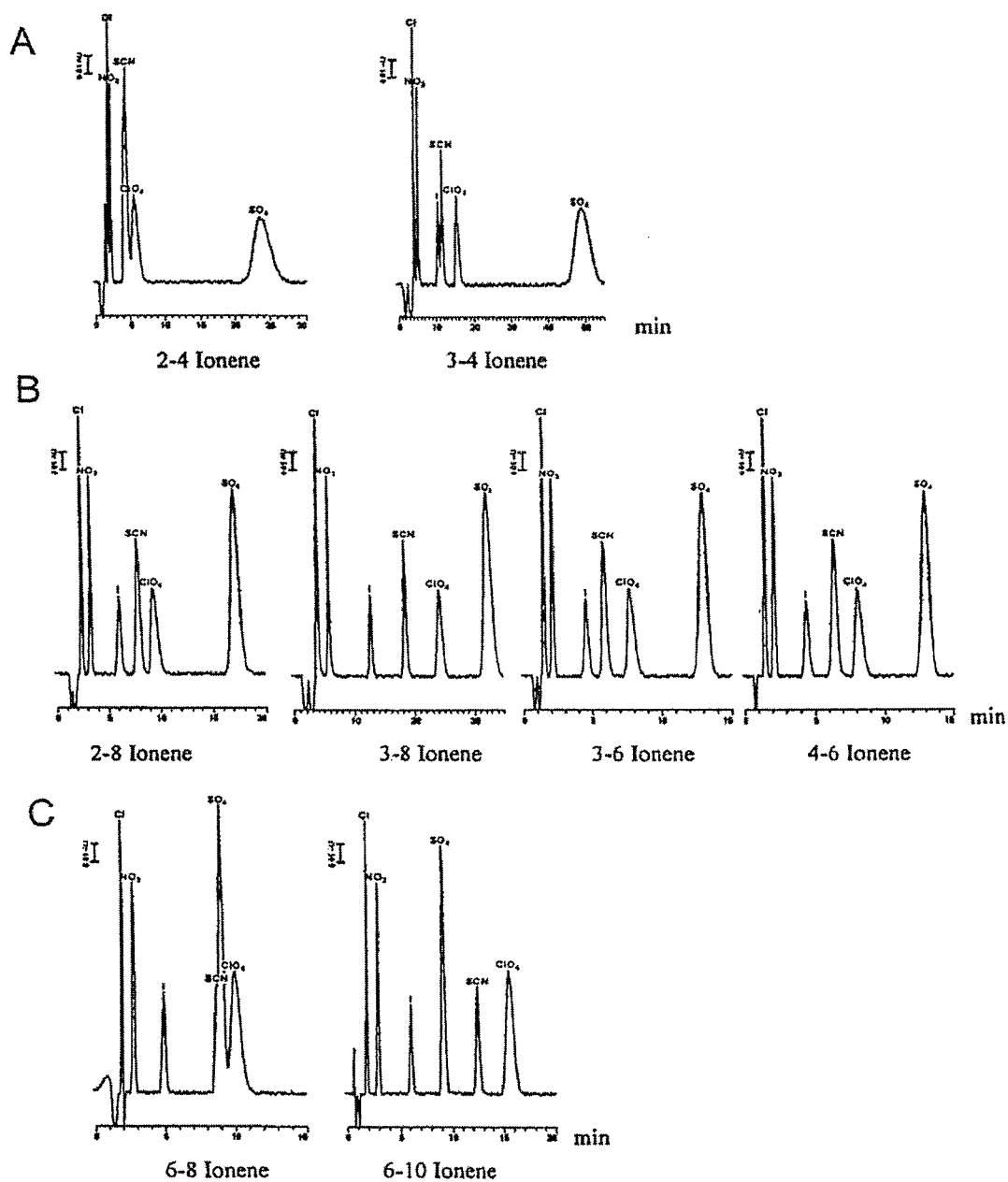


FIGURE 20. Typical ion chromatograms obtained using ionenes. Conditions: 3 × 50 mm column packed with Silasorb S; 0.3 mM potassium hydrogen phthalate eluent; pH 6.8; flow rate 1.0 mL min⁻¹; uv-detection at 254 nm. (Reprinted from *J. Chromatogr. A* 1999, 850, 53–63, with permission from Elsevier Science © 1999.)

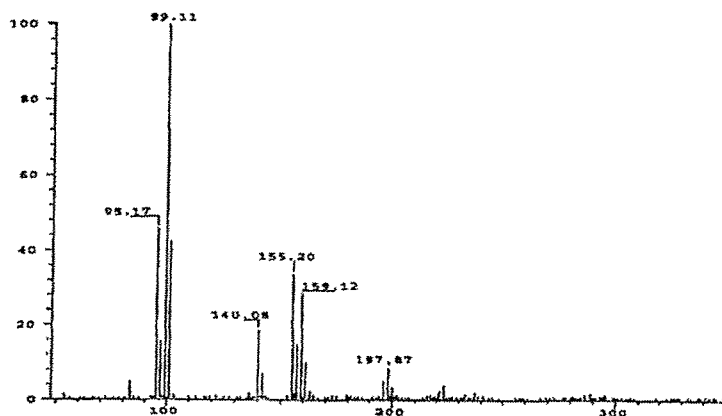


FIGURE 21. Electrospray ionization mass spectrum of perchlorate in acetonitrile/acetic acid. Molecular ions: ClO_4^- ($m/z = 99$ u), $\text{CH}_3\text{CN}\cdot\text{ClO}_4^-$ ($m/z = 140$ u), $\text{CH}_3\text{CO}_2\text{H}\cdot\text{ClO}_4^-$ ($m/z = 159$ u). (Courtesy of Air Force Research Laboratories, Wright Patterson AFB [not subject to copyright].)

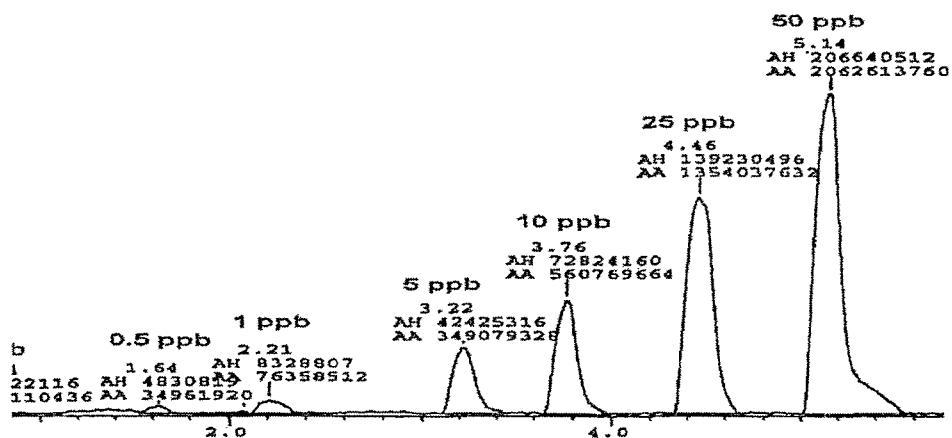


FIGURE 22. Typical ESI-MS response for perchlorate (single ion monitoring, $m/z = 99$ u) obtained in acetonitrile/acetic acid. Courtesy of Air Force Research Laboratories, Wright Patterson AFB [not subject to copyright].)

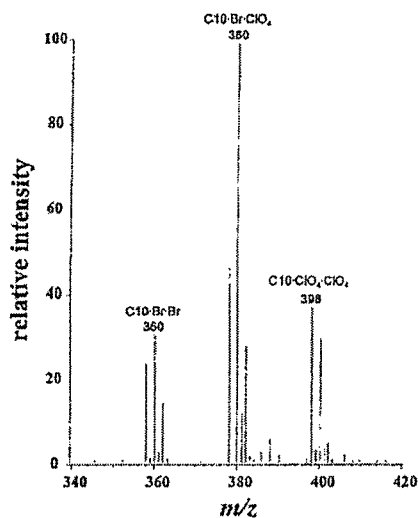


FIGURE 23. ESI mass spectrum of complex anions found when extracting perchlorate with decyltri methylammonium ion, $C_{10}H_{21}N(CH_3)_3^+$, supplied as the bromide salt. Observable complexes contain two anions. Three different anionic complexes are seen: Br/Br, Br/ ClO_4 , and ClO_4/ClO_4 . (Reprinted from *Anal. Chem.* 2000, 72, 25–29, under U.S. government authority [not subject to copyright].)

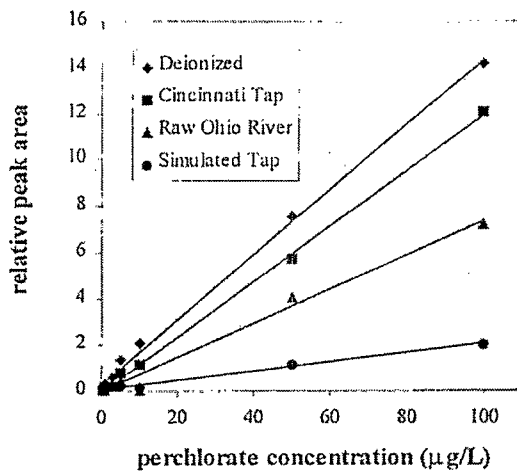


FIGURE 24. Single ion monitoring ($m/z = 380$ u) by ESI-MS for the CH_2Cl_2 -extracted complex anion $C_{10}H_{21}NMe_3(Br)(ClO_4)^-$ in several different water matrices. (Reprinted from *Anal. Chem.* 2000, 72, 25–29, under U.S. government authority [not subject to copyright].)

electrospray suppression due to ionic strength. This method has also been applied to determining perchlorate in tamarisk, a plant growing in affected regions.¹¹⁴ A tandem ESI-MS-MS system has been used for groundwater,¹¹⁵ although the equipment is not widely available.

One of the most recent developments is high field asymmetric waveform ion mobility mass spectrometry (also known as FAIMS). Barnett et al. have recently reported using FAIMS to measure the concentrations of perchlorate—even with high sulfate concentrations, but environmental samples were not specifically tested.¹¹⁶ Chlorate and bromate were readily identified by FAIMS. We can expect continuing progress from FAIMS, which appears to be capable of detection limits in the parts-per-trillion.

Although mass spectrometry is a useful research tool, it probably will not find widespread use in the drinking water industry for primary monitoring. This is primarily due to operating costs (including expertise) and instrument cost. Nonetheless, mass spectrometric methods may be expected to play important roles in confirmatory identification of perchlorate found using an IC or CE retention time.

VII. ANALYZING COMPLEX MATRICES

Although ground, surface, and treated potable water are not the simplest matrices, they are relatively easy to analyze compared with fruit juices, sludges, milk, blood, sap and other matrices likely to be of interest in assessing possible alternate exposure routes for perchlorate—not just in humans, but in animals and plants that inhabit the affected ecosystems. Our own laboratory has been investigating IC, CE, and ESI-MS for the quantitation of perchlorate in fertilizers, where it is present in trace amounts if at all, while other anions are the main components

(e.g., phosphate, nitrate, chloride).^{117–119} The EPA's National Exposure Research Laboratory has just reported a new method for the analysis of plant matter; the method focuses on substantial clean-up prior to injection.¹²⁰ The EPA has recently begun validation procedures for Method 317.0, which uses IC to separate anions, but indirect detection based on a redox reaction between bromate (or other oxyanions) and a chromophorogenic species, *o*-dianisidine (3,3'-dimethoxy-4,4'-diaminobiphenyl or 3,3'-dimethoxybenzidine).¹²¹ The reaction occurs after the separation (post-column) and is monitored photometrically. Although perchlorate reduction is kinetically hindered, it is not known if it is possible to use post-column chromogenic reaction for indirect detection of perchlorate. Because some metal ions are known to catalyze perchlorate reduction,² it may be possible to use a redox reaction by appropriate adjustment of conditions. This certainly would be an area worthy of exploration.

Analyses of more complicated (especially physiological or biological) matrices are hindered by organic anions (e.g., sulfonates, carboxylates, phenoxides), polysaccharides, fatty molecules (e.g., polyols, alkaloids, lipoproteins, phospholipids, sterols), proteins (e.g., albumin, casein). Removing these species is not necessarily straightforward. Furthermore, verifying that sample pre-treatment does not also remove the analyte will be important. At present, separation techniques such as CE and IC have been applied without extensive sample pretreatment, but fruit juices and milk are refractory to determining perchlorate when meaningful limits of detection ($<1 \mu\text{g mL}^{-1}$) are required. Online dialysis has been applied successfully to both IC and CE.¹²² It is undoubtedly a technique worthy of investigation.

In order to estimate the potential for ecological impact, it is necessary to survey representative organisms for exposure. This is where techniques amenable to single cell

sampling and analysis are useful.¹²³⁻¹²⁵ They make it possible to assess insects, annelids, helminths, nematodes, cnidarians, or small crustaceans (e.g., amphipods, isopods, cladocerans) with total body volumes of 1 to 100 mm³; these creatures can be useful indicators of water quality. In addition to making it possible to obtain a sample from organisms otherwise too small, minimizing the volume of sample needed reduces the stress on individual reptiles, fish, amphibians, birds, or small mammals, for whom even 1 mL represents a significant volume of blood. Minimizing the need for sacrificing organisms, combined with lower costs of sample collection, storage, and shipping make microanalytical techniques and methods extremely attractive for use in ecological or biological studies. Human erythrocytes have been subjected to this technique (total volume 90 fL; 1 femtoliter = 10⁻¹² L); however, enzymes have been a focus of these investigations. Intrathyroid perchlorate has not been determined this way, but perhaps it could be, that is, by harvesting individual follicular cells. EPA, USDA, and FDA have an interest in determining whether perchlorate can be transported and/or accumulated through the food chain, but thorough ecological assessments are time consuming and expensive. Therefore, measuring perchlorate at the lowest levels of the food chain is attractive in terms of saving time and money.

VIII. CONCLUSIONS

Certainly in the near future, we can expect ion chromatography to dominate environmental analytical chemistry both because of the limit of detection and the availability of the instrumentation in many laboratories. Capillary electrophoresis appears to have carved out a niche in forensic analysis and could be used in many environmental applications if sufficiently sensitive detectors were available. It is likely that Raman spectrom-

etry and mass spectrometry will be used for secondary confirmation because they rely on properties unrelated to hydrophilicity or ionic mobility, both of which influence retention/elution time. Electrochemical sensors are probably best suited to online process monitoring for quality control, but can be expected to make no inroads into environmental analysis due to their limited selectivity unless combined with separation techniques such as IC or CE. Lastly, the need to analyze complex matrices (e.g., foods, beverages, or body/plant fluids) will exact new demands on all of these techniques and will require the implementation of more sophisticated sample clean-up and pretreatment steps prior to analysis. Hyphenated techniques are apt to become important in the analysis of more complex matrices because they can improve selectivity, for example, LC-MS-MS, CE-MS, or CE-Raman spectrometry²⁹ as has been done already. In the end, the choice of techniques and methods for the quantitation of perchlorate will come down to central issues of analytical chemistry: selectivity and sensitivity. Within limits of cost and availability, whatever instrumentation can meet those needs will be pursued.

ACKNOWLEDGMENTS

The assistance of Jennifer Heffron, Raymond A. Hauck, Betty L. Merriman, and Jennie Thomas in gathering materials for this manuscript is recognized.

REFERENCES

1. Linke, S.R. *Managing Crises in Defense Industry: The PEPCON and Avtex Cases*; McNair Papers, No. 9. Institute for National Strategic Studies: Washington, D.C., 1990.
2. Urbansky, E.T. *Biorem. J.*, Perchlorate chemistry: implications for analysis and remediation. **1998**, *2*, 81-95, and references therein.

3. Urbansky, E.T.; Schock, M.R. *J. Environ. Manage.*, Issues in managing the risks associated with perchlorate in drinking water. **1999**, *56*, 79–95, and references therein.
4. Damian, P.; Pontius, F.W. *Environ. Prot.*, From rockets to remediation: the perchlorate problem. **1999**, *10*, 24–31.
5. Renner, R. *Environ. Sci. Technol.*, EPA draft almost doubles safe dose of perchlorate in water. **1999**, *33*, 110A–111A.
6. Renner, R. *Environ. Sci. Technol.* Perchlorate-tainted wells spur government action. **1998**, *32*, 210A.
7. Espenson, J.H. The Problem and Perversity of Perchlorate. In *Perchlorate in the Environment*; Urbansky, E.T., Ed. Plenum: New York: 2000; Ch. 1, and references therein.
8. Schilt, A.A. *Perchloric Acid and Perchlorates*. GFS Chemicals: Columbus, OH, 1979, and references therein. GFS advises that a 2nd edition is in preparation.
9. Clark, J.J.J. Toxicology of Perchlorate. In *Perchlorate in the Environment*; Urbansky, E.T., Ed. Plenum: New York: 2000; Ch. 3, and references therein.
10. Wolff, J. *Pharm. Revs.*, Perchlorate and the thyroid gland. **1998**, *50*, 89–105, and references therein.
11. Foye, W.O., Ed. *Principles of Medicinal Chemistry*, 3rd ed. Lea & Febiger: Philadelphia, PA, 1989; pp. 612–613.
12. Perciasepe, R. *Fed. Regist.*, Part III. Environmental Protection Agency. Announcement of the drinking water contaminant candidate list; notice. **1998**, *63* (40), 10273–10287.
13. Environmental Protection Agency. Drinking Water Contaminant Candidate List, February 1998, EPA Doc. No. 815-F-98-002.
14. Browner, C. *Fed. Regist.*, Part II. Environmental Protection Agency. 40 CFR parts 9, 141 and 142. Revisions to unregulated contaminant monitoring regulation for public water systems; final rule. **1999**, *64* (180), 50555–50620.
15. Lamb, A.B.; Marden, J.W. *J. Am. Chem. Soc.*, The quantitative determination of perchlorates. **1912**, *34*, 812–817.
16. Nabar, G.M.; Ramachandran, C.R. *Anal. Chem.*, Quantitative determination of perchlorate ion in solution. **1959**, *31*, 263–265.
17. Loebich, O. *Z. Anal. Chem.* A new method for the determination of perchloric acid (in German). **1926**, *68*, 34–36.
18. Welcher, F.J. *Organic Analytical Reagents*. Van Nostrand: New York, NY, 1947; Vol. 3, pp. 138–147.
19. Wyngaarden, J.B.; Wright, B.M.; Ways, P. *Endocrinology*, Effect of certain anions on accumulation and retention of iodide by the thyroid gland. **1952**, *50*, 537–549.
20. Collinson, W.J.; Boltz, D.F. *Anal. Chem.*, Indirect spectrophotometric and atomic absorption methods for determination of perchlorate. **1968**, *40*, 1896–1898, and references therein.
21. Weiss, J.A.; Stanbury, J.B. *Anal. Chem.*, Spectrophotometric determination of micro amounts of perchlorate in biological fluids. **1972**, *44*, 619–620. See also references therein for other methods at the time.
22. Burns, D.T.; Dunford, M.D.; Sutthivaiyakit, P. *Anal. Chim. Acta*, Spectrophotometric determination of perchlorate after extraction as protioptilinium perchlorate. **1997**, *356*, 141–143.
23. Kormosh, Z.A.; Bazel, Y.R. *J. Anal. Chem.*, Extraction of oxyanions with basic polymethine dyes from aqueous and aqueous-organic solutions: extraction-photometric determination of rhenium(VII) and tungsten(VI). **1999**, *54*, 607–611.
24. Niikura, K.; Bisson, A.P.; Anslyn, E.V. *J. Chem. Soc. Perkin Trans. 2*, Optical sensing of inorganic anions employing a synthetic receptor and ionic colorimetric dyes. **1999**, 1111–1114.
25. Ensafi, A.A.; Rezaei, B. *Anal. Lett.*, Automatic liquid-liquid extraction flow injection analysis determination of trace amounts of perchlorate with spectrophotometric detection. **1998**, *31*, 167–177.
26. Hisamoto, H.; Tohma, H.; Yamada, T.; Yamauchi, K.; Siswanta, D.; Yoshioka, N.; Suzuki, K. *Anal. Chim. Acta*, Molecular design, characterization, and application of

- multi-information dyes for multi-dimensional optical chemical sensing. Molecular design concepts of the dyes and their fundamental spectral characteristics. **1998**, 373, 271–289.
27. Drago, R.S. *Physical Methods for Chemists*, 2nd ed. Saunders: Ft. Worth, TX, 1992; Ch. 6.
 28. Eysel, H. *Spectrochim. Acta*, Raman intensities of liquids: absolute scattering activities and Cl–O bond EOPs of ClO^- , ClO_2^- , ClO_3^- , and ClO_4^- ions in aqueous solution. **1988**, 44A, 991–997.
 29. Miller, A.G.; Macklin, J.A. *Anal. Chem.*, Matrix effects on the Raman analytical lines of oxyanions. **1980**, 52, 807–812.
 30. Kowalchuk, W.K.; Walker, P.A. III; Morris, M.D. *Appl. Spectrosc.*, Rapid normal Raman spectroscopy of sub-ppm oxy-anion solutions: the role of electrophoretic preconcentration. **1995**, 49, 1183–1188.
 31. Susarla, S.; Collette, T.W.; Garrison, A.W.; Wolfe, N.L.; McCutcheon, S.C. *Environ. Sci. Technol.*, Perchlorate identification in fertilizers. **1999**, 33, 3469–3472. See also correction thereto. **2000**, 34, 224.
 32. Williams, T.L.; Martin, R.B.; Collette, T.W. *Appl. Spectrosc.*, Raman spectral analysis of perchlorate contamination in commonly-used fertilizers. **2000**, submitted for publication.
 33. Hofmeister, F. *Arch. Exp. Pathol. Pharmacol.*, Zur Lehr von der Wirkung der Salze. Über Regelmässigkeiten in der eiuweissfallenden Wirkung der Salze und ihre Beziehung zum physiologischen Verhalten derselben. **1888**, 24, 247–260.
 34. Koizumi, S.; Imato, T.; Ishibashi, N. *J. Membr. Sci.*, Bi-ionic membrane potential across a liquid anion-exchange membrane containing triphenyltin chloride. **1997**, 132, 149–158.
 35. Koizumi, S.; Imato, T.; Ishibashi, N. *Fresenius J. Anal. Chem.*, Effect of concentrations of triphenyltin chloride and anion exchanger on the anion selectivity of a plasticized PVC membrane electrode. **1997**, 357, 37–43.
 36. Ying, M.; Yuan, R.; Zhang, X.-M.; Song, Y.-Q.; Li Z.-Q.; Shen, G.-L.; Yu, R.-Q. *Analyst*, Highly selective iodide poly(vinyl chloride) membrane electrode based on a nickel(II) tetraazaannulene macrocyclic complex. **1997**, 122, 1143–1146.
 37. Volf, R.; Shishkanova, T.V.; Matijka, P.; Hamplová, M.; Král, V. *Anal. Chim. Acta*, Potentiometric anion response of poly(5,15-bis-(2-aminophenyl)porphyrin) electro-polymerized electrodes. **1999**, 381, 197–205.
 38. Zhou, Z.; Wang, Y.; Tao, J.; Fan, Y.; Wu, Y. *J. Inclusion Phenom. Mol. Recognit. Chem.*, A substituted tetraaza porphyrinogen as an electroactive component for a polymeric membrane anion-selective electrode. **1998**, 32, 69–80.
 39. Lin, X.M.; Umezawa, K.; Tohda, K.; Furuta, H.; Sessler, J.L.; Umezawa, Y. *Anal. Sci.*, Potentiometric responses of expanded porphyrin incorporated liquid membrane electrodes toward a series of inorganic and organic anions. **1998**, 14, 99–108.
 40. Li Z.-Q.; Wu, Z.-Y.; Yuan, R.; Ying, M.; Shen, G.-L.; Yu, R.-Q. *Electrochim. Acta*, Thiocyanate-selective PVC membrane electrodes based on Mn(II) complex of *N,N*-bis-(4-phenylazosalicylidene) *o*-phenylenediamine as a neutral carrier. **1999**, 44, 2543–2548.
 41. Negash, N.; Moges, G.; Chandravanshi, B.S. *Chem. Anal. (Warsaw)*, Liquid membrane electrode based on Brilliant Green-hydrogen phthalate ion pair. **1997**, 42, 579–588.
 42. Negash, N.; Chandravanshi, B.S.; Moges, G. *Bull. Chem. Soc. Ethiop.*, Ion pair extraction constants of some anions and selectivity of liquid membrane electrodes based on Crystal Violet and Brilliant Green. **1997**, 11, 93–99.
 43. Siswanta, D.; Takenaka, J.; Suzuki, T.; Sasakura, H.; Hisamoto, H.; Suzuki, K. *Chem. Lett.*, Novel neutral anion ionophores based on fluorinated (poly)ether compounds as a sensory molecule for an ion-selective electrode. **1997**, 195–196.
 44. Errachid, A.; Pérez-Jiménez, C.; Casabó, J.; Escriche, L.; Muñoz, J.A.; Bratov, A.; Bausells, J. *Sens. Actuators B*, Perchlorate-selective MEMFETs and ISEs based on a new phosphadithia macrocycle. **1997**, 43, 206–210.
 45. Ježková, J.; Musilová, J.; Vytřas, K. *Electroanalysis*, Potentiometry with perchlorate and fluoroborate ion-selective electrodes. **1997**, 9, 1433–1436.

46. Neuhold, C.G.; Kalcher, K.; Cai, X.; Raber, G. *Anal. Lett.*, Catalytic determination of perchlorate using a modified carbon paste electrode. **1996**, *29*, 1685–1704.
47. Xu, G.; Dong, S. *Electrochem. Commun.*, Electrochemiluminescent determination of perchlorate by solvent extraction in the presence of $\text{Ru}(\text{bpy})_3^{2+}$. **1999**, *1*, 463–466.
48. Grossman, P.D.; Colburn, J.C., Eds. *Capillary Electrophoresis: Theory and Practice*. Academic Press: San Diego, CA, 1992.
49. Jandik, P.; Bonn, G. *Capillary Electrophoresis of Small Molecules and Ions*. VCH: New York, NY, 1993. Perchlorate is specifically mentioned on pages 160 and 261.
50. Baker, Dale R. *Capillary Electrophoresis*. Wiley-Interscience: New York, NY, 1995. Perchlorate is specifically mentioned on page 43.
51. Li, S.F.Y. *Capillary Electrophoresis: Principles, Practice and Applications*. Elsevier: Amsterdam, the Netherlands, 1992.
52. Nann, A.; Pretsch, E. *J. Chromatogr. A*, Potentiometric detection of anions separated by capillary electrophoresis using an ion-selective electrode. **1994**, *676*, 437–442.
53. Ehmann, T.; Bächman, K.; Fabry, L.; Rüfer, H.; Pahlke, S.; Kotz, L. *Chromatographia*, Optimization of the electrokinetic sample introduction in capillary electrophoresis for the ultra trace determination of anions on silicon wafer surfaces. **1997**, *45*, 301–311.
54. Hargadon, K.A.; McCord, B.R. *J. Chromatogr.*, Explosive residue analysis by capillary electrophoresis and ion chromatography. **1992**, *602*, 241–247.
55. Kishi, T.; Nakamura, J.; Arai, H. *Electrophoresis*, Application of capillary electrophoresis for the determination of inorganic ions in trace explosives and explosive residues. **1998**, *19*, 3–5.
56. Warren, D.; Hiley, R.W.; Phillips, S.A.; Ritchie, K. *Sci. Justice*, Novel technique for the combined recovery, extraction and clean-up of forensic organic and inorganic trace explosives samples. **1999**, *39*, 11–18.
57. Park, S.-W.; Jin, K.-H.; You, J.-H.; Kim, T.-J.; Paeng, K.-J.; Kong, K.-H. *Anal. Sci.*, Analysis of inorganic anions by capillary zone electrophoresis. **1997**, *13*, 243–246.
58. Xiong, X.; Li, S.F.Y. *J. Chromatogr. A*, Design of background electrolytes for indirect photometric detection based on a model of sample zone absorption in capillary electrophoresis. **1999**, *835*, 169–185.
59. Xiong, X.; Li, S.F.Y. *Electrophoresis*, Dual UV-absorbing background electrolytes for simultaneous separation and detection of small cations and anions by capillary zone electrophoresis. **1998**, *19*, 2243–2251.
60. Kitagawa, S.; Tsuji, A.; Watanabe, H.; Nakashima, M.; Tsuda, T. *J. Micro. Sep.*, Pressurized flow-driven capillary electrochromatography using ion exchange resins. **1997**, *9*, 347–356.
61. Biesaga, M.; Kwiatkowska, M.; Trojanowicz, M. *J. Chromatogr. A*, Separation of chlorine-containing anions by ion chromatography and capillary electrophoresis. **1997**, *777*, 375–381.
62. Doble, P.; Macka, M.; Haddad, P.R. *J. Chromatogr. A*, Use of dyes as indirect detection probes for the high-sensitivity determination of anions by capillary electrophoresis. **1998**, *804*, 327–336.
63. Harrold, M.P.; Wojtusik, M.J.; Riviello, J.; Henson, P. *J. Chromatogr.*, Parameters influencing separation and detection of anions by capillary electrophoresis. **1993**, *640*, 463–471.
64. Shamsi, S.A.; Danielson, N.D. *Anal. Chem.*, Naphthalenesulfonates as electrolytes for capillary electrophoresis of inorganic anions, organic acids, and surfactants with indirect photometric detection. **1994**, *66*, 3757–3764.
65. Krokhin, O.V.; Hoshino, H.; Shpigun, O.A.; Yotsuyanagi, T. *J. Chromatogr. A*, Use of cationic polymers for the simultaneous determination of inorganic anions and metal-4-(2-pyridylazo)resorcinolato chelates in kinetic differentiation-mode capillary electrophoresis. **1997**, *776*, 329–336.
66. Stathakis, C.; Cassidy, R.M. *Can. J. Chem.*, Control of relative migration of small inorganic and organic anions with cyclodextrins in capillary electrophoresis (CE). **1998**, *76*, 194–198.
67. Avdalovic, N.; Pohl, C.A.; Rocklin, R.D.; Stillian, J.R. *Anal. Chem.*, Determination of

- cations and anions by capillary electrophoresis combined with suppressed conductivity detection. **1993**, *65*, 1470–1475.
68. Gross, L.; Yeung, E.S. *J. Chromatogr.*, Indirect fluorimetric detection and quantification in capillary zone electrophoresis of inorganic anions and nucleotides. **1989**, *480*, 169–178.
 69. Macka, M.; Gerhardt, G.; Andersson, P.; Bogan, D.; Cassidy, R.M.; Haddad, P.R. *Electrophoresis*, Capillary electrophoresis with end-capillary potentiometric detection using a copper electrode. **1999**, *20*, 2539–2546.
 70. Kappes, T.; Schnierle, P.; Hauser, P.C. *Anal. Chim. Acta*, Potentiometric detection of inorganic anions and cations in capillary electrophoresis with coated-wire ion-selective electrodes. **1997**, *350*, 141–147.
 71. Corr, J.J.; Anacleto, J.F. *Anal. Chem.*, Analysis of inorganic species by capillary electrophoresis–mass spectrometry and ion exchange chromatography–mass spectrometry using an ion spray source. **1996**, *68*, 2155–2163.
 72. Hsu, J.-C.; Chen, W.-H.; Liu, C.-Y. *Analyst*, Preparation of a macrocyclic polyamine-bonded column for the electrophoretic separation of inorganic and organic anions. **1999**, *122*, 1393–1398.
 73. Hauser, P.C.; Hong, A.P.C.; Renner, N. *J. Cap. Elec.*, Surface charge reversal for inorganic anion determination in capillary electrophoresis with an ion-selective microelectrode as detector. **1995**, *2*, 209–212.
 74. Hilder, E.F.; Macka, M.; Haddad, P.R. *Anal. Commun.*, Mixed-mode capillary electrochromatographic separation of anionic analytes. **1999**, *36*, 299–303.
 75. Meissner, T.; Eisenbeiss, F.; Jastorff, B. *J. Chromatogr. A*, New leading electrolyte for the direct determination of chloride and other anions in analytical isotachophoresis. **1999**, *838*, 81–88.
 76. Small, H. *Ion Chromatography*. Plenum Press: New York, NY, 1990.
 77. Okada, T. *J. Chromatogr. A*, Nonaqueous anion-exchange chromatography. I. Role of solvation in anion-exchange resin. **1997**, *758*, 19–28.
 78. California Department of Health Services, Sanitation and Radiation Laboratories Branch. *Determination of Perchlorate by Ion Chromatography*, Rev. 0. Jun. 3, 1997.
 79. Okamoto, H.S.; Rishi, D.K.; Steeber, W.R.; Baumann, F.J.; Perera, S.K. *J. Am. Water Works Assoc.*, Using ion chromatography to detect perchlorate. **1999**, *91*, 73–84.
 80. Miura, Y.; Kimura, M.; Ochi, M.; Koh, T. *Proc. – School Sci., Tokai Univ.*, Effect of eluents on suppressed ion chromatography using anion exchange column of Dionex HPIC-AS5. **1996**, *31*, 83–90.
 81. Maurino, V.; Minero, C. *Anal. Chem.*, Cyanuric acid-based eluent for suppressed anion chromatography. **1997**, *69*, 3333–3338.
 82. Wirt, K.; Laikhtman, M.; Rohrer, J.; Jackson, P.E. *Am. Environ. Lab.*, Low level perchlorate analysis in drinking water and ground water by ion chromatography. **1998**, *10*, 1, 5.
 83. Jackson, P.E.; Laikhtman, M.; Rohrer, J. *Proc. – Water Qual. Technol. Conf.*, An improved chromatographic method for the determination of trace level perchlorate in drinking water and ground water. AWWA: San Diego, CA, Nov. 1–4, 1998, 2058–2065.
 84. Jackson, P.E.; Laikhtman, M.; Rohrer, J.S. *J. Chromatogr. A*, Determination of trace level perchlorate in drinking and ground water by ion chromatography. **1999**, *850*, 131–135.
 85. Jackson, P.E.; Gokhale, S.; Rohrer, J.S. Recent Developments in the Analysis of Perchlorate Using Ion Chromatography. In *Perchlorate in the Environment*; Urbansky, E.T., Ed. Kluwer/Plenum: 2000; Ch. 5.
 86. Harrington, P.; Shen, Y. *Proc. – Water Qual. Technol. Conf.*, Improved analysis of perchlorate in water. AWWA: San Diego, CA, Nov. 1–4, 1998, 1405–1412.
 87. Nair, L.M.; Saari-Nordhaus, R.; Montgomery, R.M. *J. Chromatogr. A*, Applications of a new methacrylate-based anion stationary phase for the separation of inorganic anions. **1997**, *789*, 127–134.
 88. Eaton, A.; Haghani, A.; Cox, N.; Wong, E. *Proc. – Water Qual. Technol. Conf.*, A comparison of the ruggedness of different analytical methods for perchlorate. AWWA: San Diego, CA, Nov. 1–4, 1998, 1413–1423.
 89. Tsui, D.T.; Clewell, R.A.; Eldridge, J.E.; Mattie, D.R. Perchlorate Analysis with the

- AS16 Column. In *Perchlorate in the Environment*; Urbansky, E.T., Ed. Kluwer/Plenum: 2000; Ch. 7.
90. Hautman, D.P.; Munch, D.J.; Eaton, A.D.; Haghani, A.W. Method 314.0. Determination of Perchlorate in Drinking Water Using Ion Chromatography, Revision 1.0. Environmental Protection Agency: Cincinnati, OH, 1999. EPA Doc. No. 815-B-99-003.
 91. Buchberger, W.; Haider, K. *J. Chromatogr. A*, Studies on the combination of ion chromatography-particle-beam mass spectrometry with capillary columns. **1997**, 770, 59-68.
 92. Daignault, L.G.; Rillema, D.P.; Jackman, D.C. *J. High Resol. Chromatogr.*, Retention behavior of ions on high performance ion chromatography columns. Part II: Retention behavior and polarizability. **1990**, 13, 293-294.
 93. Atkins, P.W. *Molecular Quantum Mechanics*, 2nd ed. Oxford University Press: Oxford, UK, 1983; p. 364.
 94. Huheey, J.E.; Keiter, E.A.; Keiter, R.L. *Inorganic Chemistry: Principles of Structure and Reactivity*, 4th ed. Harper-Collins: New York, NY, 1993; p. 299.
 95. Martin, M.W. *Microchem. J.*, Selectivity of some ion chromatography stationary phases for small anions in solvent-water mixtures with hydroxide. **1999**, 62, 203-222.
 96. Okada, T. *J. Chromatogr. A*, Interpretation of chromatographic behavior of ions based on the electric double-layer theory. **1999**, 850, 3-8.
 97. Watanabe, T.; Kubota, M. *Anal. Chim. Acta*, Electrostatic effect of streaming potential on ion-exchange phenomena in resins for ion chromatography. **1990**, 22, 61-67.
 98. Pirogov, A.V.; Obrezkov, O.N.; Shpigun, O.A. *J. Anal. Chem.*, Temperature as a parameter of optimization of the ion chromatographic determination of anions. **1997**, 52, 152-156.
 99. Takeuchi, T.; Suzuki, E.; Ishii, D. *Chromatographia*, Ion chromatography with micropacked alumina columns. **1988**, 25, 480-482.
 100. Elfakir, C.; Chainbault, P.; Dreux, M. *J. Chromatogr. A*, Determination of inorganic anions on porous graphitic carbon using evaporative light scattering detection. Use of carboxylic acids as electronic competitors. **1998**, 829, 193-199.
 101. Muentner, M.M.; Stokes, K.C.; Obie, R.T.; Jezorak, J.R. *J. Chromatogr. A*, Simultaneous separation of inorganic ions and neutral organics on ion-exchange stationary phases. **1999**, 844, 39-51.
 102. Kocsis, E.M.; Koizummi, S.; Sakane, T.; Takahashi, K.; Imato, T. *Anal. Sci.*, Separation behavior of anions on ODS modified with phthalocyanine cobalt(III) complex. **1997**, 13, 195-198.
 103. Umemura, T.; Kamiya, S.; Itoh, A.; Chiba, K.; Haraguchi, H. *Anal. Chim. Acta*, Evaluation of sulfobetaine-type zwitterionic stationary phases for ion chromatographic separation using water as a mobile phase. **1997**, 349, 231-238.
 104. Pirogov, A.V.; Platonov, M.M.; Shpigun, O.A. *J. Chromatogr. A*, Polyelectrolyte sorbents based on aliphatic ionenes for ion chromatography. **1999**, 850, 53-63.
 105. Snyder, A.P. ACS Symposium Series 619: *Biochemical and Biotechnological Applications of Electrospray Ionization Mass Spectrometry*. American Chemical Society: Washington, DC, 1995.
 106. Watson, J.T. *Introduction to Mass Spectrometry*, 3rd ed. Lippincott-Raven: Philadelphia, 1997.
 107. Barnett, D.A.; Horlick, G. *J. Anal. At. Spectrom.*, Quantitative electrospray mass spectrometry of halides and halogenic anions. **1997**, 12, 497-501.
 108. Clewell, R.E.; Tsui, D. *Prepr. Ext. Abstr. ACS Natl. Meet., Am. Chem. Soc. Div. Environ. Chem.* Presented at the 218th American Chemical Society national meeting, New Orleans, LA, USA, Aug. 22-26, **1999**, 39, 71-74.
 109. Clewell, R.; Chaudhuri, S.; Dickson, S.; Cassady, R.S.; Wallner, W.M.; Eldridge, J.E.; Tsui, D. Analysis of Trace Level Perchlorate in Drinking Water and Ground Water by Electrospray Mass Spectrometry. In *Perchlorate in the Environment*; Urbansky, E.T., Ed. Kluwer/Plenum: 2000; Ch. 6.

110. Urbansky, E.T.; Magnuson, M.L.; Freeman, D.; Jelks, C. *J. Anal. At. Spectrom.* Quantitation of perchlorate ion by electrospray ionization mass spectrometry (ESI-MS) using stable association complexes with organic cations and bases to enhance selectivity. **1999**, *14*, 1861–1866.
111. Magnuson, M.L.; Urbansky, E.T.; Kelty, C.A. *Anal. Chem.*, Determination of perchlorate at trace levels in drinking water by ion-pair extraction with electrospray ionization mass spectrometry. **2000**, *72*, 25–29.
112. Magnuson, M.L.; Urbansky, E.T.; Kelty, C.A. *Talanta*, Microscale extraction of perchlorate in drinking water with low level detection by electrospray-mass spectrometry. **2000**, *52*, 285–291.
113. Urbansky, E.T.; Gu, B.; Magnuson, M.L.; Brown, G.M.; Kelty, C.A. *J. Sci. Food Agric.*, Survey of bottled waters for perchlorate by electrospray ionization mass spectrometry (ESI-MS) and ion chromatography (IC). **2000**, *80*, 1798–1804.
114. Urbansky, E.T.; Magnuson, M.L.; Kelty, C.A.; Brown, S.K. *Sci. Tot. Environ.*, Perchlorate uptake by salt cedar (*Tamarix ramosissima*) in the Las Vegas Wash riparian ecosystem. **2000**, *256*, 227–232.
115. Koester, C.J.; Beller, H.R.; Halden, R.U. *Environ. Sci. Technol.*, Analysis of perchlorate in groundwater by electrospray ionization mass spectrometry/mass spectrometry. **2000**, *34*, 1862–1864.
116. Handy, R.; Barnett, D.A.; Purves, R.W.; Horlick, G.; Guevremont, R. *J. Anal. At. Spectrom.*, Determination of nanomolar levels of perchlorate in water by ESI-FAIMS-MS. **2000**, *15*, 907–911.
117. Urbansky, E.T.; Brown, S.K.; Magnuson, M.L.; Kelty, C.A. *Environ. Pollut.*, Distribution of perchlorate in samples of sodium nitrate fertilizer derived from Chilean caliche. **2000**, *110*, in press.
118. Urbansky, E.T.; Gu, B.; Furman, C.S.; Magnuson, M.L.; Brown, G.M.; Kelty, C.A. Quantitation of perchlorate ion in fertilizers: a comparison of ion chromatography, electrospray ionization mass spectrometry, and capillary electrophoresis. In preparation.
119. Urbansky, E.T.; Gu, B.; Magnuson, M.L.; Brown, G.M.; Kelty, C.A. *Environ. Sci. Technol.*, Comment on 'perchlorate identification in fertilizers.' **2000**, *34*, in press.
120. Ellington, J.J.; Evans, J.J. *J. Chromatogr. A*, Determination of perchlorate at parts-per-billion levels in plants by ion chromatography. **2000**, in press.
121. Wagner, H.P.; Pepich, B.V.; Hautman, D.P.; Munch, D.J. Determination of Inorganic Oxyhalide Disinfection Byproducts in Drinking Water Using Ion Chromatography with the Addition of a Postcolumn Reagent for Trace Bromate Analysis. Environmental Protection Agency: Cincinnati, OH, 1999; Draft Method 317.0.
122. Kuban, P.; Karlberg, B. *Anal. Chem.*, On-line dialysis coupled to a capillary electrophoresis system for determination of small anions. **1997**, *68*, 1169–1173, and references therein.
123. Hogan, B.L.; Yeung, E.S. *Trends Anal. Chem.*, Single-cell analysis at the level of a single human erythrocyte. **1993**, *12*, 4–9.
124. Sims, C.E.; Meredith, G.D.; Krasieva, T.B.; Berns, M.W.; Tromberg, B.J.; Allbritton, N.L. *Anal. Chem.*, Laser-micropipet combination for single-cell analysis. **1998**, *70*, 4570–4577.
125. Swanek, F.D.; Ferris, S.S.; Ewing, A.G. Capillary Electrophoresis for the Analysis of Single Cells: Electrochemical, Mass Spectrometric, and Radiochemical Detection. In *Handbook of Capillary Electrophoresis*, 2nd ed.; Landers, J.P., Ed. CRC Press: Boca Raton, FL, 1997; 495–521.
126. Lillard, S.J.; Yeung, E.S. Capillary Electrophoresis for the Analysis of Single Cells: Laser-Induced Fluorescence Detection. In *Handbook of Capillary Electrophoresis*, 2nd ed.; Landers, J.P., Ed. CRC Press: Boca Raton, FL, 1997; 523–544.